MAY 25 1923
Medical Library

VOLUME 31 NUMBER 5

ARCHIVES
OF
INTERNAL MEDICINE

EDITORIAL BOARD
JOSEPH L. MILLER, Chicago
RICHARD C. CABOT, Boston
LOUIS V. HAMMAN, Baltimore
GEORGE DOCK, Pasadena, Calif.
WARFIELD T. LONGCOPE, Baltimore
W. S. THAYER, Baltimore

MAY 15, 1923

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION, $5.00

Entered as Second-Class Matter, January 23, 1909, at the Postoffice at Chicago, Illinois, Under Act of Congress of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on June 14, 1918.

CONTENTS OF PREVIOUS NUMBERS

FEBRUARY, 1923. NUMBER 2

Protein Feeding and High Blood Pressure. Solomon Strouse, M.D., and Sarah R. Kelman, M.D., Chicago.

Relation Between Hemoglobin, Cell Count and Cell Volume in the Venous Blood of Normal Human Subjects. H. C. Gram, M.D., and A. Norgaard, M.D., Copenhagen.

Toxic Manifestations Following the Alkaline Treatment of Peptic Ulcer. Leo L. Hardt, M.D., and Andrew B. Rivers, M.D., Rochester, Minn.

A Simple Immersion Electrode for Taking Clinical Electrocardiograms. Harold E. B. Pardee, M.D., New York.

Paroxysmal Ventricular Tachycardia: Report of One Case with Normal Type of Auricular Mechanism and Three with Auricular Fibrillation. C. C. Wolferth, M.D., and Thomas M. McMillan, M.D., Philadelphia.

The Surgical Treatment of Angina Pectoris. Walter B. Coffey, M.D., and Philip King Brown, M.D., San Francisco.

Fatty Degeneration of the Heart. A. M. Master, M.D., New York.

Intrapericardial Rupture of Aortic Aneurysm in a Boy Sixteen Years of Age. DeWayne G. Richey, Pittsburgh.

Blood Sugar Standards. Part I. Normal and Diabetic Persons. Horace Gray, M.D., Boston.

Blood Sugar Standards. Part II. In Conditions Neither Normal nor Diabetic. Horace Gray, M.D., Boston.

Intracutaneous Reactions in Lobar Pneumonia to Pneumotoxin. (A Reply to Dr. George H. Bigelow.) Charles Weiss, Ph.D., and John A. Kolmer, M.D., Philadelphia.

Some Observations on the Value of the Index and Angle of Bordet-Vaquez in Cardiac Examination. David S. Dunn, M.D., Boston.

Pathologic Physiology of Polycythemia Vera. Raphael Isaacs, M.D., Cincinnati.

The Etiology of Acute Intestinal Intoxication in Infants. Gladys L. Boyd, M.B., Toronto.

Book Review.

MARCH, 1923. NUMBER 3

Further Observations on the Roentgen-Ray Treatment of Toxic Goiter. J. H. Means, M.D., and G. W. Holmes, M.D., Boston.

The Correlation of Vital Capacity with Stem Height. William Lester Rogers, San Francisco.

A Clinical Report on the Use of Quinidin Sulphate. L. E. Viko, M.D.; H. M. Marvin, M.D., and Paul D. White, M.D., Boston.

Enlargement of Hemal Nodes. Max Lederer, M.D., Brooklyn, N. Y.

Studies on the Detoxification of Cyanids: I. Some Factors Influencing the Detoxification of Cyanids in Health and Disease. Meyer Bodansky, M.A., and Moise D. Levy, M.D., Galveston, Tex.

Studies of the Blood Gases in a Case of Paroxysmal Tachycardia. Edward P. Carter, M.D., and Harold J. Stewart, M.D., Baltimore.

Observations of the Cardiovascular System in Thyroid Disease. W. J. Kerr, M.D., and George C. Hensel, M.D., San Francisco.

A Case of Rheumatic Fever with Cerebral Symptoms Simulating Encephalitis Lethargica. Lewis Fux Frissell, M.D., New York.

Gravimetric Estimation of Phosphates of the Blood: Phosphates in Nephritis. William J. Fetter, M.D., Pittsburgh.

The Increase of Certain Vagal Effects with Increased Age. N. C. Gilbert, M.D., Chicago.

Studies of Exophthalmic Goiter and the Involuntary Nervous System. Leo Kessel, M.D.; Charles C. Lieb, M.D., and Harold T. Hyman, M.D. III. A Study of Fifty Consecutive Cases of Exophthalmic Goiter. Leo Kessel, M.D.; Harold T. Hyman, M.D., and Herman Lande, M.D., New York.

APRIL, 1923. NUMBER 4

Further Observations on the Use of a High Fat Diet in the Treatment of Diabetes Mellitus. L. H. Newburgh, M.D., and Phil L. Marsh, M.D., Ann Arbor, Mich.

A Stem-Stature Index. H. Gray, M.D., Boston.

Albuminuria: Its Clinical Significance as Shown by Chemical Study of the Blood. T. Homer Coffen, M.D., Portland, Ore.

Clinical Experience with Quinidin. C. Sidney Burwell, M.D., and Francis R. Dieuaide, M.D., Baltimore.

Mycotic (Bacterial) Aneurysms of Intravascular Origin. Alfred Stengel, M.D., and C. C. Wolferth, M.D., Philadelphia.

Distribution of Sugar in Whole Blood, Plasma and Corpuscles; Permeability of Red Blood Corpuscles for Sugar in Diabetic and Non-diabetic Cases. Henry J. John, M.D., Cleveland.

A Study of Blood Pressure in Relation to Types of Bodily Habitus. Joseph W. Larimore, M.D., St. Louis.

Hemoglobinuria in Hemolytic Jaundice. Herbert Z. Giffin, M.D., Rochester, Minn.

The Relation of Age and Obesity to Vital Capacity. Byron D. Bowen, M.D., with the Assistance of Dorothy L. Platt, Buffalo, N. Y.

Acid-Base Equilibrium. I. Clinical Studies in Alkalosis. Alfred E. Koehler, Madison, Wis.

Physical and Chemical Studies of Human Blood Serum. I. Study of Normal Subjects. Dana W. Atchley, Robert F. Loeb, Ethel M. Benedict and Walter W. Palmer, New York.

Physical and Chemical Studies of Human Blood Serum. II. A Study of Twenty-Nine Cases of Nephritis. Dana W. Atchley, Robert F. Loeb, Ethel M. Benedict and Walter W. Palmer, New York.

Physical and Chemical Studies of Human Blood Serum. III. A Study of Miscellaneous Disease Conditions. Dana W. Atchley, Robert F. Loeb, Ethel M. Benedict and Walter W. Palmer, New York.

Book Review.

Copyright, 1923, by the American Medical Association

Archives of Internal Medicine

VOL. 31 MAY, 1923 No. 5

OBSERVATIONS ON THE VALUE OF PHENOLTETRACHLORPHTHALEIN IN ESTIMATING LIVER FUNCTION *

GEORGE MORRIS PIERSOL, M.D., AND H. L. BOCKUS, M.D.
PHILADELPHIA

In 1913, Rowntree, Hurwitz and Bloomfield,[1] in making a résumé of all known methods for testing the functional capacity of the liver concluded that they were all of little value in that they did not deal with functions specific to the liver. They made the possible exception of the urobilinogen test, which they found positive in most mild diseases of the liver, but it gave no information as to the extent of liver injury, as it entirely lacked the quantitative side. It was of no value, therefore, from the standpoint of prognosis.

During the course of a pharmacologic search for a cathartic of protracted action, it was discovered by Rowntree and Abel[2] in 1909, that phenoltetrachlorphthalein was eliminated entirely by the liver, and they described its properties. They found it to be an odorless, tasteless chrystalline substance, insoluble in water and forming deeply colored hydrolizable salts with alkalies. They found its avidity as an acid not far removed from phenolphthalein. Its ionization constant has not been determined. The dye was first prepared by Orndorff and Black[3] in 1908.

Rowntree and his associates first advocated the use of tetrachlorphthalein to test the functional capacity of the liver. They injected 8 c.c. (400 mg.) of the dye diluted to 100 c.c. with physiologic solution of sodium chlorid intravenously by the gravity method. Catharsis was instituted at 6 a. m. with Epsom salts and the dye injected at noon,

---

* From the Gastro-enterological Clinic of the Graduate School of Medicine of the University of Pennsylvania.

* Read before the Association of American Physicians, Washington, D. C., May 4, 1922.

1. Rowntree, Hurwitz and Bloomfield: Experimental and Clinical Study of the Value of Phenoltetrachlorphthalein as a Test for Liver Function, Bull. Johns Hopkins Hosp. **23**:327, 1913.

2. Abel, J. J., and Rowntree, L. G.: On the Pharmacological Action of Some Phthaleins and Their Derivatives, with Especial Reference to Their Action as Purgatives, J. Pharmacol. & Exper. Therap. **1**:231, 1909.

3. Orndorff, W. R., and Black, J. A.: Phenoltetrachlorphthalein and Some of Its Derivatives, Am. Chem. J. **41**:349, 1909.

followed by cathartic pills, if necessary. Little attention was paid to the diet. Stools were collected for forty-eight hours following the injection. The dye was separated from the stool, and the amount excreted was estimated in the Rowntree-Geraghty modification of the Autenreith Koenigsberger colorimeter. They experimented on dogs with biliary fistulas and found that the dye appeared free in the bile in fifteen minutes following its intravenous administration, and that in forty-eight hours, from 35 to 55 per cent. of it could be recovered in the feces. Their studies indicated that the excretion of tetrachlor would be useful in the estimation of the functional capacity of the liver.

Whipple, Pughtal and Clark [4] showed that the normal output of dye in the feces of dogs was remarkably constant, and that the drop in tetrachlor output ran parallel to the amount of liver injury. Passive congestion of the liver of moderate degree caused little impairment of function and showed a normal output of dye. They found that destruction of liver tissue by the actual cautery caused a prompt drop in the tetrachlor output. Delay of dye excretion from such an injury was present but could not be demonstrated in work with the feces. They found the urine of dogs free from dye or, at most, only a trace (pink on adding alkali), following the injections.

Sisson [5] found the normal output of dye in the feces to be about 35 per cent. He reached no definite conclusion as to the clinical value of the test.

Chesney, Marshall and Rowntree [6] found the lower limit of dye elimination in the feces to be 30 per cent. of the amount injected in forty-eight hours. The dye was never found in the urine in health after the administration of 400 mg., according to these observers.

Krumbhaar,[7] in 1914, in summarizing the status of functional liver tests, concludes that although no satisfactory single test for the functional capacity of the liver has yet been accepted, the tetrachlor test of Rowntree promises the greatest value. McLester and Frazier,[8] in 1915, following Rowntree's technic, concluded that "this test taken alone in its present form is of no value clinically." Kahn and Johnston,[9] in

4. Whipple, Pughtal and Clark: Tests for Hepatic Function and Disease Under Experimental Conditions, Bull. Johns Hopkins. Hosp. **24**:343, 1913.
5. Sisson, W. R.: A Clinical Study of Two Hepatic Functional Tests, Arch. Int. Med. **14**:804 (Dec.) 1914.
6. Chesney, A. M.; Marshall, E. K., and Rowntree, L. G.: Studies in Liver Function, J. A. M. A. **63**:1533 (Oct. 31) 1914.
7. Krumbhaar, E. B.: Present Status of Functional Liver Tests, New York M. J. **100**:719 (Oct. 10) 1914.
8. McLester, J. S., and Frazier, B.: Phenoltetrachlorphthalein Test for Liver Function, J. A. M. A. **65**:385 (July 31) 1915.
9. Kahn, M., and Johnston, J. B.: The Phenoltetrachlorphthalein Test for Liver Function, New York M. J. **102**:848, 1915.

1915, following the same technic, reached a similar conclusion after studying a series of thirty-four cases.

In 1916, McNeil [10] modified the Rowntree method by inserting the duodenal tube into the duodenum and injecting 400 mg. of the dye. He recorded the time of first appearance of the dye in the bile. The bile was obtained by aspiration. He collected all the bile excreted in two hours in this way and estimated the percentage recovered. The difficult and cumbersome technic of the feces method and the conflicting opinions regarding its value, stimulated him in the study. He found that the dye first appeared in the bile normally (six cases) in from twelve to twenty-one minutes and that the total amount recovered through the duodenal tube in two hours varied from a trace to 10 per cent. The greater part of the dye was excreted during the first hour. The first appearance time of the dye in five cases of Laennec's cirrhosis varied from twenty-eight to forty-five minutes. He found a normal output of dye in three of these cases and practically no dye was recovered in two cases. He concluded that the dye elimination was of little value, but that its appearance time was decidedly delayed in certain pathologic conditions of the liver. Kahn,[11] in 1921, modified this procedure somewhat by stimulating the flow of bile into the duodenum. He administered a solution of magnesium sulphate and aspirated every ten minutes.

Aaron, Beck and Schneider [12] modified the duodenal technic and introduced a stable preparation of the dye. They believe that the quantitative estimation of the dye excreted is of little value, owing to the uncertainty of the quantity passed out through the bowel. Consequently, they were satisfied with estimating the time of appearance of the maximum color. They made it possible to get a much more reliable appearance time of the dye by establishing a drip from the tube. This was accomplished by the administration of 500 c.c. of water by mouth and injection of the dye after a steady drip from the tube was established. They reported 17.2 minutes as the average appearance time of the maximum color in the bile in sixteen normal cases, whereas the average in six pathologic cases was thirty-two minutes.

Summarizing the work which has been done, we conclude that the study of Rowntree, Hurwitz and Bloomfield, and of Whipple, Pughtal

10. McNeil, H. L.: The Quantitative Estimation of Phenoltetrachlorphthalein Excreted in the Fresh Bile in Disease of the Liver, J. Lab. & Clin. M. **1**:822, 1915.

11. Kahn, M.: Phenoltetrachlorphthalein Test on the Duodenal Content, J. A. M. A. **77**:41 (July 6) 1921.

12. Aaron, A. H.; Beck, E. C., and Schneider, H. C.: The Phenoltetrachlorphthalein Test for Liver Function, J. A. M. A. **77**:1631 (Nov. 19) 1921.

and Clark carried out both clinically and experimentally have proved the following:

1. That since phenoltetrachlorphthalein is eliminated solely by the bile in health, it makes an ideal substance for testing the function of the liver.

2. That the forty-eight hour output of dye in the feces is fairly constant in health.

3. That the time of appearance of the dye in the bile is of decided importance, but it cannot be determined by the feces method.

4. That the dye output decreases as the hepatic parenchymal damage becomes more extensive. Chesney et al. and Krumbhaar confirmed their clinical findings, whereas Sisson, McLester and Frazier, and Kahn and Johnston concluded that the test was of no clinical value.

In view of the difficulty of carrying out the test and the lack of sufficient clinical confirmation it has not been widely adopted. The more recent work of McNeil, Aaron et al. with the duodenal tube has, we believe, opened up a new field for investigation. We were stimulated to go on with this work and attempt to arrive at some definite conclusion, feeling that in view of the demonstrated merit of tetrachlor, a technic could be developed which would make this test in some measure comparable to the phenolsulphonephthalein kidney test. After following Aaron's technic in a few cases, it was decided to enlarge on it in order to gather a maximum amount of information. The series presented includes fifty cases, normal and pathologic. The data recorded include the appearance time of the first faint pink, the appearance time of the maximum color and the number of milligrams of dye eliminated by the liver and obtained from the duodenum during a period of two hours when collected at half hourly intervals.

It is interesting to note that in a number of normal cases, after the test was completed, the urine failed to show the presence of any of the dye.

OUTLINE OF PROCEDURE

1. The duodenal tube (any type of tube) is passed into the stomach, so that the tip is at a point approximately twenty-one inches from the lips. It is introduced in the morning on a fasting stomach for two reasons: (*a*) the tube passes through the pylorus more quickly on the morning fasting stomach; (*b*) the liver is always in the same physiologic condition at this time, its digestive function not being exercised.

2. The stomach is washed by giving two glasses of water by mouth and aspirating until clean. Sixty c.c. of water is introduced into the stomach to be retained.

3. The patient is turned on the right side and the tube is very slowly pushed downward by the patient until the tip is at a point twenty-seven inches from the lips. Lyon's method was used, allowing twenty minutes for the introduction of the tube to this point, the patient being instructed to breathe deeply and swallow slowly. If the tube does not pass the pylorus, it is pulled out to the twenty-one inch mark and the procedure is repeated. The passage of the tube into the duodenum rarely takes longer than one hour, barring organic obstruction, adhesions, persistent pylorospasm, or severe degrees of atony or ptosis. To ascertain whether or not the tip of the tube is in the duodenum does not present difficulty. The following points indicate its presence in the duodenum: (*a*) Presence of the duodenal tug, i. e., if the tube is in the duodenum, the plunger of the aspirating syringe will return to its previous position when traction is made on it. It pulls out easily, as a rule, when the tube is in the stomach. The water left in the stomach can usually be aspirated if this is the case. An exception occurs to this rule, if the tube is kinked in the stomach. (*b*) Appearance of pure yellow alkaline bile on aspiration. (*c*) Appearance of golden yellow froth. (*d*) Failure to obtain water in the syringe on aspirating after half a glass has been taken by mouth. (*e*) Lyon's stethoscope method. (*f*) Thirty c.c. of warm water allowed to run into the duodenum by gravity, the tube lowered and water syphoned back. If the tube is in the duodenum, the water will return bile stained. This procedure opens the sphincter of Oddi and causes bile to be expelled. This is probably the most reliable method when all others fail. It has not been necessary to resort to the fluoroscope. If the procedures are carried out in the order as given above, a definite decision as to the location of the tip can usually be made.

4. Just as soon as the tube enters the duodenum, 500 c.c. of water is given by mouth. The dye is not injected until a steady drip of bile stained fluid is coming from the tube. This step, introduced by Aaron and his co-workers, is an important one.

5. One hundred and fifty mg. of the disodium salt of phenoltetrachlorphthalein as prepared in ampouls by Hynson, Westcott and Dunning (each c.c. containing 50 mg.) is injected into a vein and the time of injection is recorded.

6. The bile is allowed to drip out into a white basin containing 3 or 4 c.c. of a 40 per cent. solution of sodium hydroxid.

7. The time of appearance of the first faint pink color is recorded. The syringe is not to be used. If it is necessary to aspirate, the appearance time cannot be considered reliable. Aspirating every ten minutes, as advocated by Kahn, would render the appearance time of little value,

as a delay of more than nine minutes might thus escape recognition. Kahn suggested the use of magnesium sulphate to stimulate the flow of bile; this is unnecessary when the water is given by mouth. As Einhorn has shown, a solution of magnesium sulphate when introduced into the duodenum stimulates the liver, but the question at issue now is how the liver functions under normal conditions and not when under the influence of a stimulant.

8. The appearance time of the maximum color, which is a deep purple, is recorded.

9. Each half hour's output is allowed to drip into a separate basin. Each basin must contain not more than 5 c.c. of a 40 per cent. sodium hydroxid solution. It is important to avoid using an excess of alkali as it makes the bile pigments more difficult to precipitate. In addition it gives the solution a reddish tint which cannot be compared with the standard dye. The time of injection was taken as the starting point of the two hour period, and not time of first appearance of the dye.

10. A continuous slow drip from the tube should be maintained throughout the two hour interval. This can be accomplished by having the patient drink a glass of water every half hour.

To estimate the amount of dye eliminated, the procedure is as follows: In the absence of a suitable colorimeter, we made up standard solutions containing a known amount of dye dissolved in 1,000 c.c. of water; 100 c.c. of each of these known solutions of the dye was placed in a thoroughly washed 100 c.c. bottle and tightly stoppered. The bottles used as standards represent the following amounts of dye in mg. 0.1, 0.2, 0.3, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5 and 20, respectively. These solutions will remain practically unchanged for from four to six weeks, when a slight precipitate forms, due to the alkali of the disodium salt uniting with the silicate of the glass bottle.

Each half hour's output is run through as follows: (*a*) Pour the sample of dye laden bile into a 1,000 c.c. stoppered graduate. Add sufficient 40 per cent. sodium hydroxid solution to bring out the maximum intensity of color. Avoid an excess because it makes it more difficult to precipitate out the bile pigments. If this occurs a few drops of nitric acid should be added and the fluid realkalized with hydroxide until the color appears again. Dilute to 1,000 c.c. and shake. (*b*) Measure out 100 c.c. of this solution in a graduate and add about 3 c.c. of a saturated solution of basic lead acetate and 2 c.c. of a saturated solution of calcium chlorid. The acetate solution precipitates the bile pigment and the calcium chlorid seems to assist particularly in those specimens which contain a considerable quantity of bile. (*c*) Pour into a 100 c.c. test tube and heat in a water bath until the pre-

cipitate is being agitated. This requires but a few minutes. (*d*) Filter to remove the precipitate. (*e*) Pour into a bottle similar to those containing the standard and make comparative readings.

Dr. Edgar N. Cowan of the department of Biochemistry of the Graduate School of Medicine of the University of Pennsylvania, kindly worked out this method of precipitating the bile coloring matter.

Of the fifty subjects studied, thirty-five suffered from a great variety of clinical conditions. Fifteen had normal livers. In order better to appreciate the significance of the results, they have been tabulated into four groups (Tables 1, 2, 3 and 4).

Table 1.—As far as can be ascertained clinically, these subjects have normal livers. Three of them were tested before the technic for the

TABLE 1.—OUTPUT, 19 MG. OR MORE; FIFTEEN CASES

Name	Age	Diagnosis	First Appearance Time of Dye, Min.	Time of Maximum Intensity of Color, Min.	Output in Mg. First Half Hour	Output in Mg. Second Half Hour	Output in Mg. Third Half Hour	Output in Mg. Fourth Half Hour	Total Output in Mg.	Time of Total Output, Hours
Pic.	21	Normal	10	11	10.0	6.2	7.5	1.5	25.2	2
Bis.	51	Normal	8	11	4.0	10.0	2.0	8.0	24.0	2
Bar.	38	Normal	11	13	4.0	6.0	6.0	3.0	19.0	2
Dro.	30	Normal	8	11	8.0	12.0	1.5	0.0	21.5	2
Ros.	22	Normal	11	14	6.5	5.0	....	....	11.5	1
Gre.	37	Normal	9.5	15						
Kran.	55	Normal	8	9.2						
Amb.	40	Chr. cholecystitis	9	14						
Darl.	52	Chr. cholecystitis	7	9	3.7	4.5	15.0	0.15	23.35	2
Kraf.	65	Chr. cholecystitis	10.5	15	9.0	3.0	....	....	12.0	1
Mel.	34	Chr. cholecystitis	7.5	10.5	9.0	5.0	2.0	3.0	19.0	2
Aur.	17	Chr. appendicitis	7	8.75	6.0	10.0	6.0	5.0	27.0	2
Go.	27	Psychoneurosis	10	13	1.5	1.0	15.0	2.5	20.0	2
S. B.	30	Gastric neurosis	7	11	2.0	12.5	2.0	4.0	20.5	2
A. S.	36	Pernicious anemia. R. B. C., 4,000,000	5	9	9.0	10.0	2.5	3.0	24.5	2
Average	36		8.56	11.56	6.05	7.1	5.95	3.01	22.4	2

quantitative estimation was perfected. They were placed in this group because their livers were clinically negative and their dye appearance time more closely conformed to the average time appearance for this group. In two of the cases the output for the first hour only was taken. Their first hour output corresponds to the average first hour elimination for this group. Seven of the fifteen cases had no disease or their diagnosis was undetermined. None of them had fever or any indication of toxemia. In four cases a diagnosis of chronic cholicystitis had been made. There was one case each of chronic appendicitis, psychoneurosis, gastric neurosis and pernicious anemia. The patient with pernicious anemia was in good condition. His red cell count was 4,000,000 as he had had a transfusion recently. The ages of this group ranged from 17 to 60 years. The youngest, a girl of 17, had the highest

output of dye (27 mg.). A young man of 21 was next with an output of 25.2 mg. The average elimination for two hours was 22.5 mg., the output ranging from 19 to 27 mg. The average elimination for the first hour was 13.1 mg., and for the second hour 8.9 mg. The greatest output of dye occurred during the first hour in health. The first appear-

TABLE 2.—Output, from 10 to 19 Mg.; Seventeen Cases

Name	Age	Diagnosis	First Appearance Time of Dye, Min.	Time of Maximum Intensity of Color, Min.	Output in Mg. First Half Hour	Output in Mg. Second Half Hour	Output in Mg. Third Half Hour	Output in Mg. Fourth Half Hour	Total Output in Mg.	Time of Total Output, Hours
Shee.	50	Hypertrophic cirrhosis; liver 4 fingers below costal margin; chronic cholecystitis	9.5	19.5	1.5	3.0	5.0	0.5	10.0	2
For.	53	Congestion of liver; edge 2 fingers below costal margin; myocarditis	11	16	3.0	8.0	1.0	0.5	12.5	2
Wag.	50	Severe diabetes; liver to umbilicus	14	14	2.0	5.0	2.0	1.5	10.5	2
Res.	72	Diabetes, gangrene, chronic nephritis	8.5	11.5	8.0	2.0	1.0	0.5	11.5	2
Con.	40	Diabetes..... ..	12	16	0.5	4.5	1.0	5.0	11.0	2
Wal.	63	Arteriosclerosis....	13	17						
Piel.	64	Moderate arteriosclerosis	12.5	13.5						
Fra.	30	Cholecystitis; chr. appendicitis; pulmonary tuberculosis	14							
Snel.	66	Chr. cholecystitis; cholelithiasis; periduodenal adhesions	25	26	4.0	7.0	0.75	0.1	11.85	2
Gre.	50	Chr. cholecystitis..	8.5	14	6.0	5.0	2.0	2.0	16.0	2
Koe.	52	Carcinoma of stomach; severe secondary anemia; liver neg.	7	10	6.0	1.5	2.5	4.0	14.0	2
Brin.	47	Osteo-arthritis.....	8	12	5.0	3.0	2.5	3.0	13.5	2
P. O. H.	53	Acute multiple arthritis	9	10	2.0	2.0	6.0	1.0	11.0	2
S. L.	26	Convalescent pneumonia	12	16	2.5	2.0	0.75	9.0	14.25	2
Mor.	36	Chronic catarrhal sigmoiditis	8	10	6.5	3.0	1.0	0.5	11.0	2
Rich.	38	Chronic ulcerative colitis	10	16	10.0	0.25	0.25	0.0	10.5	2
Mak.	31	Multiple sclerosis (5 years)	9.0	15	4.5	4.0	1.5	1.5	11.5	2
Average..	48	.....	11.2	14.8	4.39	3.58	1.9	2.15	12.79	2

ance time varied from five to eleven minutes with an average of 8.6 minutes. The appearance time of the maximum intensity of the dye varied from 8.75 to 15 minutes, with an average of 11.6 minutes. The first appearance time in McNeil's series of five normal cases was from twelve to twenty-one minutes. We believe his later appearance time may be explained by two factors: (1) use of the gravity method

of administering the dye, and (2) unreliability of the aspiration method. In certain cases when the sphincter of Oddi is not open, the aspiration method may give erroneous results as pointed out above in discussing the technic.[9] McNeil emphasizes the fact that the entrance of bile into the duodenum is controlled by contraction of the gallbladder, and that some cases of supposedly delayed excretion time might be due to failure of the gallbladder to contract. The contraction of the gallbladder is not necessary in order to obtain the flow of bile into the duodenum. Almost any solution poured into the duodenum will cause an opening of the sphincter and a pouring out of bile even in the absence of a gallbladder. It is not necessary to obtain gallbladder bile in this test, although we believe that a variable amount is usually expelled. The administration of water by mouth, thus insuring a steady drip from the tube, is enough to keep the bile flowing.

Aaron et al. report 17.2 minutes as the average maximum color appearance in sixteen normal cases, using 75 mg. of dye, as compared to our average of 11.6 minutes with 150 mg. of dye. We feel that this latter amount of dye should be used when a quantitative estimation is to be carried out, as it is easier to work with larger amounts of dye in making comparisons with the standards.

Table 2.—This includes seventeen cases in which the elimination is from 10 to 19 mg. Three of these cases have only the appearance time recorded. They were classified here because the time of appearance of the dye was similar to the average for this group. Three patients had definitely enlarged livers. Their dye output was very nearly identical, averaging 11 mg. (50 per cent. of the normal). The output of dye in three diabetics averaged only 11 mg., there being but 1 m., difference between the extreme figures (10.5; 11.5 mg.). Seven cases can be classified as chronic infectious processes, in any one of which the liver might easily become involved secondarily. This subgroup includes one case each of infectious arthritis, osteo-arthritis, chronic catarrhal sigmoiditis, and chronic ulcerative colitis, and one case of chronic cholelithiasis with obstruction and periduodenal adhesions. However, there was nothing which clinically suggested hepatic insufficiency. It is in this type of case in which the liver is clinically negative, that the test should be of particular value. There is one case each of multiple sclerosis, convalescent pneumonia and carcinoma of the stomach in this group. The average first appearance time is 11.2 minutes as compared to 8.6 minutes of the normal group. The average maximum color time is 14.8 minutes as compared with 11.6 minutes of the normal group. The average dye output is 12.79 mg.; that for the normal group is 22.9 mg.

Table 3.—This includes eight cases in which the elimination is from 5 to 10 mg. Three cases of advanced cardiovascular disease in which a diagnosis of liver disease had not been made show a distinctly lessened dye output and a slightly delayed appearance time. A definite diagnosis of cirrhosis of the liver had been made on another. Still another presented active manifestations of tertiary syphilis but no evidence of liver disturbance. One patient was convalescing from a

TABLE 3.—OUTPUT, FROM 5 TO 10 MG.; EIGHT CASES

Name	Age	Diagnosis	First Appearance Time of Dye, Min.	Time of Maximum Intensity of Color, Min.	Output in Mg. First Half Hour	Output in Mg. Second Half Hour	Output in Mg. Third Half Hour	Output in Mg. Fourth Half Hour	Total Output in Mg.	Time of Total Output, Hours
Hib.	62	General arteriosclerosis; cardiac hypertrophy; chr. endocarditis; coronary sclerosis; syphilis	11	16	2.5	2.5	0.75	1.5	7.25	2
Jack.	45	General arteriosclerosis; aortic regurgitation	8	15	2.0	1.0	....	....	3.0	1
Mars.	68	Aortitis; Cor bovinum; enlarged mediastinal lymph nodes; cholelithiasis (no obstruction), acute attacks; no HCl	10	13	2.0	2.0	1.0	1.0	6.0	2
Con.	32	Cirrhosis of liver; syphilis	12	18	3.2	0.1	2.0	2.5	7.6	2
W. H.	36	Tertiary syphilis, Wassermann ++++	10	14	2.0	1.0	1.5	0.6	5.1	2
Arm.	31	Convalescent pneumonia; erysipelas; nonhemolytic streptococcus bacteremia	7	14.5	0.1	2.0	1.75	3.0	6.85	2
Eisn.	30	Convalescent; operation for chr. appendicitis; chr. pelvic disease; gastric neurosis, vagotonia	11	11	7.0	2.0	0.75	0.0	9.75	2
Yan.	15	Colonic stasis.....	11	24.5	2.5	2.0	0.2	1.5	6.2	2
Average..	40	.....	9.87	15.75	2.66	1.57	1.13	1.44	6.96	2

severe streptococcus pneumonia, which may account for his low dye output. The other two patients were suffering from chronic infectious conditions of the abdomen. Whether these infections were severe enough to cause liver disturbance we could not determine. The average first appearance time was 9.87 minutes as compared to 8.6 of the normal group. The average maximum color time was 15.75 minutes as compared with 11.6 minutes in the normal group. The average dye output was 6.96 mg.; that for the normal group was 22.4 mg.

Table 4.—This table includes ten cases in which the elimination is under 5 mg. In this group we have placed patients whose clinical diagnoses include liver incompetency and whose dye output was less than 5 mg. (under 25 per cent. of normal). Three of them had far advanced cardiovascular disease, one had portal cirrhosis and another had a moderately congested liver. In three of them the delayed appear-

TABLE 4.—OUTPUT, LESS THAN 5 MG.; TEN CASES

Name	Age	Diagnosis	First Appearance Time of Dye, Min.	Time of Maximum Intensity of Color, Min.	Output in Mg. First Half Hour	Output in Mg. Second Half Hour	Output in Mg. Third Half Hour	Output in Mg. Fourth Half Hour	Total Output in Mg.	Time of Total Output, Hours
Pem.	60	Convalescent acute nephritis; myocarditis; chr. cholecystitis; bile and urine cultures; staphylococcus aureus and albus	24.5	29.5	1.0	2.0	0.3	0.0	3.3	2
Dro.	49	Arteriosclerosis; aortic aneurysm; mitral stenosis	13	17	0.5	0.4	0.2	0.4	1.5	2
Har.	53	Arteriosclerosis; cardiac hypertrophy; chronic nephritis	14	24	0.1	1.5	0.3	0.0	1.9	2
Adley....	64	Portal cirrhosis; aortitis	16	26	1.0	1.5	0.3	0.0	2.8	2
Hol.	37	Distorted duodenum from periduodenal adhesions; cholecystitis, proved by operation	10	19	0.1	1.0	3.0	0.0	4.1	
E. B.	50	Cholecystitis (stones); hepatitis; moderate congestion	11.5	25						
Boa.	39	Cholelithiasis (partial obstruction); operated, bladder full of stones, 10 in common duct	13	21.5	1.5	1.5	0.0	0.0	3.0	2
Rob.	33	Partial common duct obstruction	15	None						
W. Sal. ..	62	Cholelithiasis; obstruction common duct; small amount of bile	11	23	0.5	0.4	0.75	0.75	2.4	2
Sall.	36	Tabes; questionable syphilitic hepatitis	8	14	0.5	1.1	....	....	1.6	1
Average..	**48**	.....	13.6	23.22	0.65	1.17	0.69	0.16	2.71	2

ance time and decreased dye output was due to common duct obstruction. Naturally, one gathers no information as to the functional capacity of the liver in such cases. In another case the lessened dye extraction was due, at least in part, to a grossly distorted duodenum from adhesions. A case of tabes had a dye output of only 1.6 mg. in one hour. This man had a four plus Wassermann and a questionable syphilitic hepatitis. Thirteen and six-tenths minutes was the average

first appearance time for this group as compared with 8.6 minutes in the normal group. The average maximum appearance time was 11.6 minutes in the normal group as compared to the 23.22 minutes in this group. The average dye output was 2.71 mg. as compared with 22.4 mg.

Table 5.—This table shows at a glance the hourly excretions. There was so much individual variation in the half hourly output, that we feel it is of little clinical significance. In each group, however, the average output for the first hour was greatly in excess of that for the second hour. In certain individual instances in both normal and pathologic subjects, the second hour's output was in excess of that

TABLE 5.—COMPARISON BETWEEN FIRST AND SECOND HOUR OUTPUTS OF THE FOUR GROUPS

Group	Number of Cases	Output, Mg.	First Hour Average, Mg.	Second Hour Average, Mg.
1	15	19 up	13.15	8.96
2	17	10-19	8.97	4.05
3	8	5-10	4.23	2.57
4	10	Under 5	1.82	0.85

TABLE 6.—COMPARISON BETWEEN THE APPEARANCE TIME OF THE DYE AND THE QUANTITATIVE EXCRETION OF DYE IN TWO HOURS

Group	Number of Cases	Average Excretion for Group, Mg.	Average First Appearance Time of Dye, Min.	Average Maximum Color Time, Min.	Extremes in First Appearance Time in Each Group, Min.	Extremes in Appearance Time of Maximum Color in Each Group, Min.
1	15	22.4	8.6	11.6	5 to 11	8.7 to 15
2	17	12.79	11.2	14.8	7 to 25	10 to 26
3	8	6.96	9.87	15.75	7 to 12	11 to 24.5
4	10	2.71	13.6	23.22	8 to 24.5	14 to 34

for the first hour. McNeil's observation that in diseased livers the greatest output of dye occurred in the second hour is not confirmed by this investigation. A great many more pathologic livers will have to be studied before any definite conclusion can be reached.

Table 6.—Here we have tabulated the average dye output and appearance time for each group. In a general way, the smaller the amount of dye excreted, the slower it is in making its appearance. At least, this is true for the appearance of the maximum intensity of color. The latter appears to be of more significance than the time of the appearance of the first faint pink color. It will be noticed that in Group 2 the dye is slower in making its appearance than in Group 3, whereas the dye output in Group 2 is twice that in Group 3. This is not true of the appearance time of the maximum color. The maximum color

is twice as long in making its appearance in Group 4 as it is in Group 1, whereas the difference is not nearly as marked in the first appearance time. We have tabulated the minimum and maximum figures of appearance time in each group (Table 6). It will be noticed that the range of variation is extensive. For this reason we believe the time of appearance of the dye is of less importance than the estimation of the quantitative output over the period of two hours. The average dye output in Group 4 is less than one eighth of the output in the normal group, whereas the average maximum color appearance time in Group 4 is only twice as great as the normal figure. Considerable variation in the appearance time obviously may depend on the personal equation of the observer as well as upon technical difficulties, such as the temporary plugging of the tube with mucus. For these reasons we believe that the quantitative estimation of the dye output is of more significance than merely recording the appearance time.

In considering the value of estimating the quantitative output of dye when obtained through the duodenal tube, the question arises as to how much comes out through the tube and what quantity passes on down through the bowel. With the patient in the recumbent position, either on the right side or the back (preferably the right side) the second or descending portion of the duodenum is the most dorsal part of the gut. As pointed out by Knight, it constitutes a U-shaped trap, with the second part of the duodenum at the bottom of the U. It is this portion of the intestine which fills rapidly but empties slowly. Into this portion, about four inches from the pylorus, the bile empties. The tip of the tube should be passed down to a point twenty-seven inches from the lips. If the tube is passed to this point it will rest in the U-shaped trap. Care should be taken to keep the tube in this position throughout the examination. The amount of dye and bile obtained in normal individuals with thoroughly patent duct systems was found to be so nearly constant that we feel the quantitative estimation of the dye output is of decided importance.

CONCLUSIONS

1. We have presented here a technic in detail which it is believed will yield information of decided value concerning the functional competency of the liver.

2. Owing to the fact that the duodenal tube is now being used universally, this test can be added to the armamentarium of the average clinic with ease.

3. When a larger number of cases are accumulated and our normal standards become more definite, it should be comparable to the phenolsulphonephthalein kidney function test, as there seems no good reason

for believing that the liver reserve is any greater than that of the kidney when these two organs are compared as to size and their relative importance in the body economy.

4. In a series of fifty cases, we found that, in a general way, a delay in the appearance time of the dye was proportionate to a decrease in the output. The estimation of the dye output in two hours is of more importance than recording the appearance time, but we believe both should be considered.

5. In grossly pathologic livers the appearance time of the maximum color was twice as long as that in normal cases (23.2 minutes as compared to 11.6 minutes) and the dye output averaged but one eighth of the output in normal cases (2.71 mg. as contrasted with 22.4 mg.).

6. We suspect that the output of dye in normal cases will vary with the age, the younger the subject, the greater the amount of dye which should be covered.

7. Lastly, and most important, we believe that in this test we have a method of learning something of the functional capacity of the liver when it is but slightly disturbed and when it is clinically negative to other methods of examination. It is in this group of cases particularly that the estimation of the quantitative two hour output will be of more assistance than that of the appearance time of the dye.

THE HEART IN DIPHTHERIA

A CLINICAL AND PATHOLOGIC STUDY *

MATHILDE LOTH, M.D.

NEW HAVEN, CONN.

Cardiac failure has long been recognized as a cause of death in diphtheria. It is the factors which produce this death and the mechanism of the process that have caused dispute and discussion. Cardiac failure has been attributed by the majority of observers to an injury to the myocardium itself or its conducting system, but others have thought that the disturbance was extracardiac, that is, in the peripheral vasomotor system. On account of the various conflicting theories, it may be well to review briefly some of the opinions held respecting this very important phenomenon, before presenting our own observations.

Coghlan [1] believes that the diphtheria toxin acts directly on the heart muscle, resulting in certain degenerative changes, and that this is the cause of the circulatory failure. Farr [2] carries this conception further, expressing the view that the diphtheria toxin has a peculiar affinity for heart muscle, the extrinsic and intrinsic cardiac nerves, and the blood vessels. Fleming and Kennedy [3] cite a case of complete heart block in which the necropsy showed a definite myocarditis with inflammatory foci in the conducting bundle. These authors state that the case demonstrates that death in diphtheria, when the heart rate is slow and when signs of palatal paralysis are present, is not necessarily the result of inhibitory cardiac impulses.

Romberg, Pässler, Brühns and Rolly believe that early death in diphtheria is due to paralysis of the vasomotor centers in the medulla, and that late death is referable to an interstitial myocarditis. Concerning this point of early and late death, Holt [4] says that the explanation of heart failure during or after diphtheria is not always the same. When it occurs at the height of the disease, it is sometimes due to coronary thrombosis, probably always associated with changes in

* From the Department of Pathology and Bacteriology, Yale University School of Medicine.

1. Coghlan, E. F.: The Management of Cardiac Failure in Diphtheria, Brit. M. J. **1**:534, 1912.

2. Farr, C. B.: The Significance of Arrhythmia in Infections of Childhood, with Special Reference to Diphtheria, Penn. M. J. **23**:633, 1920.

3. Fleming, G. B., and Kennedy, A. M.: A Case of Complete Heart Block in Diphtheria, with an Account of Postmortem Findings, Heart **2**:77, 1910.

4. Holt, L. E.: Diseases of Infancy and Childhood, New York, D. Appleton & Co., 1920, pp. 835-1038.

the myocardium. When it occurs late, and follows some sudden muscular effort or excitement without premonitory symptoms of any sort, it is probably the result of a true myocarditis. He adds, "It is by no means certain that cardiac paralysis is due to a lesion of the cardiac nerves. Toxic Myocarditis appears to be a more important factor in producing the fatal result."

Experiments made by MacCallum [5] on dogs, in which the influence of the vasomotors was almost entirely eliminated, tended to prove that the actual work of the diphtheria poisoned heart was fully as good as that of the normal heart. MacCallum concludes that death occurring at the height of an attack of diphtheria is not exclusively the result of direct injury to the heart, although damage to this organ may play some part in the process.

Brodie,[6] who sought to find the cause of early death in diphtheria, that is, death within forty-eight hours after the onset of the disease, concludes from his experiments on cats that up to a very short time before death both the blood vessels and the heart are apparently in a nearly normal condition; then there is a sudden fall in blood pressure, death following in from ten to fifteen minutes. The fall is due to the relaxation of the blood vessels, the heart continuing to beat strongly to the end. He concludes that the chief cause of death within forty-eight hours is "failure of the blood vessels, and consequent fall in blood pressure."

Price and Mackenzie [7] state that "cardiac paralysis" in diphtheria has long been recognized as being associated, in the majority of cases, with extensive myocarditis. Rohmer [8] has studied cases which show that diphtheria toxin has no special affinity for the conducting system. Tanaka [9] agrees that lesions of the conducting system are certainly very rarely the cause of cardiac death. Abramow [10] believes that subacute death is due to regressive changes in the heart muscle which come about

5. MacCallum, W. G.: The Mechanism of the Circulatory Failure in Diphtheria, Am. J. M. Sc. **147**:37, 1914.

6. Brodie, T. G.: The Physiological Action of Diphtheria Toxin, Brit. M. J. **2**:128, 1899.

7. Price, F. W., and Mackenzie, I.: Auricular Fibrillation and Heart Block in Diphtheria, Heart **3**:233, 1911.

8. Rohmer, P.: Elektrocardiographische und anatomische Untersuchungen über den Diphtherie-Herztod und dessen Beziehungen zum Reizbeitungssystem, Ztschr. f. exper. Path. und Therap. **11**:426, 1912.

9. Tanaka, T.: Ueber die Veränderungen der Herzmuskultur, vor allem des Atrioventrikular-bündels bei Diphtherie; zugleich ein Beitrag zur Frage der Selbständigkeit des Bündels, Virchows Arch. f. path. Anat. **207**:115, 1912.

10. Abramow, S.: Pathologish-anatomische Studien über experimentelle Diphtherie-intoxikation und Diphtherie immunität, Ztschr. f. Immunitätsforsch. u. exper. Therap. **15**:12, 1912.

from the lack of epinephrin, since under the influence of large doses of diphtheria toxin the suprarenal secretion fails.

From this brief résumé it will be seen that there is by no means unanimity of opinion as to the cause of cardiac failure in diphtheria.

Although, as this résumé shows, there is great divergence of opinion as to the exact nature of the circulatory failure, there is not so much disagreement among clinicians and pathologists as to the clinical and pathological phenomena associated with the condition.

Clinically, the reported cardiac findings in cases of diphtheria have been fairly uniform. Flexner [11] in 1895, quoted figures of G. Hoppe-Seyler, which indicate that the percentage of cases in which the heart is affected in the course of diphtheria varies from 10 to 38. Richardson,[12] in an analysis of one hundred deaths from diphtheria, states that in forty per cent. of the cases "myocarditis" was a complication. According to Hitchcock's observations, cardiac complications were the cause of death in 15 per cent. of deaths in a large epidemic of diphtheria. According to Osler,[13] irregularity of the heart is common, being present in 60 per cent. of the cases. Murmurs at the apex or base of the heart are present in 94 per cent. of all cases. Only a relatively small percentage of cases of diphtheria show serious heart symptoms.

The conditions which are regarded as serious are rapid heart action with gallop rhythm, and a drop in the pulse from 110 to 30 or 40. The physical signs include a feeble apex beat, outside the nipple line and diffuse in nature, an increase of the cardiac area to the right, to the left, or to both sides, heart sounds which are difficult to hear, murmurs, usually soft, at both apex and base, and a gallop rhythm. The pulse shows great variations; it may be full and bounding, though it is usually feeble, with diminished tension. S. Calvin Smith [14] points out that the pulse abnormalities can be divided into initial tachycardia and the irregularities of convalescence. Initial tachycardia is of serious import only when it persists during convalescence, and it may then be regarded as the probable precursor of heart block. Fifteen per cent. of the convalescent irregularities consist of high grade heart block, which is strikingly sudden in onset; death, therefrom, may be expected within

11. Flexner, S.: Bacteriology and Pathology of Diphtheria, Am. J. M. Sc. **109**:240, 1895.

12. Richardson, D. L.: Analysis of One Hundred Deaths from Diphtheria, Rhode Island M. J. **3**:87, 1920.

13. Osler, William, and McCrae, T.: Principles and Practice of Medicine, 1920, p. 780.

14. Smith, S. C.: Observations on the Heart in Diphtheria, J. A. M. A. **77**:765 (Sept. 3) 1921.

thirty-six hours; none of the patients in the series of 242 patients recovered from this condition. McCulloch,[15] from a study of eighty cases of diphtheria, concludes that in those cases in which the effect on the heart is so profound as to interfere with cardiac mechanism, the outcome is usually fatal. In his study, the mortality rate was 100 per cent. Dilatation of the heart is frequently described, and Hecht [16] who confirmed his diagnosis by the use of the roentgen ray, was able to demonstrate dilatation in the roentgenogram in 25 per cent. of cases.

However, it is through the interpretation of the electrocardiogram that the most definite evidence of myocardial involvement in diphtheria is being found today. Almost every type of arrhythmia has been observed. Hume and Clegg [17] have reported auricular premature beats, ventricular premature beats, paroxysmal tachycardia, auricular flutter, auricular fibrillation, heart block, nodal premature beats and reversal of nodal beats.

Aviragnet and Lutembacher [18] maintain that the arrhythmia depends on the degree of toxic impregnation and disappears with it, but then emphasize the well known fact that tachycardia often persists for weeks and months. They describe a case with complex extrasystolic arrhythmia with paroxysmal tachycardia and deranged conduction in a boy, aged 10, with ultimate recovery. According to Farr, the arrhythmias which occur most commonly in diphtheria are sinus irregularity, heart block, and premature contractions. He adds that in children sinus arrhythmia has no pathological significance, certainly not as regards the heart. He also remarks that auricular fibrillation is not common in diphtheria; it may be temporary, though it is usually permanent and indicates serious myocardial weakness and exhaustion.

From the clinical point of view, the pathologic interpretation of these arrhythmias is a matter of especial interest. Do any definite lesions underly these irregularities? Blacher [19] says that the appearance of gallop rhythm and extrasystoles is not a direct index of the degree of anatomical damage in the myocardium, although gallop rhythm and myocarditis often occur together. Coghland believes that the presence of extrasystoles, with or without consecutive intermission of the heart's

15. McCulloch, H.: Effect of Diphtheria on the Heart, J. A. M. A. **77**:765 (Sept. 3) 1921.

16. Hecht, A. F.: Der Mechanismus der Herzaktion im Kindesalter, seine Physiologie und Pathologie, Ergebnisse d. inn. Med. u. Kinderh. **2**:324, 413, 1913.

17. Hume, W. E., and Clegg, S. J.: A Clinical and Pathological Study of the Heart in Diphtheria, Quart. J. M. **8**:1, 1914-1915.

18. Aviragnet, E. C., and Lutenbacher, R.: Le Coeur dans la Diphtérie, Arch. d. mal. du coeur **13**:1, 197, 1920.

19. Blacher, W.: Galopprhythmus und extrasystolen bei der diphtherischen Myokarditis, Jahrb. f. Kinderh. **29**:166, 1914.

action, is important evidence that the heart wall is involved and that reduplication of the first sound followed by accentuation of the second is a sign of advanced myocardial disease. Aviragnet thinks that premature contractions may be provoked by slight myocardial lesions which are purely irritative. Hume and Clegg state that any form of arrhythmia of the heart, except sinus arrhythmia, indicates in diphtheria that the heart or nerves are involved in a pathological process, however mild the illness may appear. Rupe's [20] idea is that often lesions of the myocardium cause no clinical symptoms, but invariably the slightest lesions of the bundle produces marked clinical manifestations of conduction changes.

That lesions of the conducting tissue are not regularly present in cases of clinically demonstrable irregularity, has been demonstrated by the observations of Aviragnet, Lutembacher and LeSoudier,[21] and others. Furthermore, Lewis and Mathison have shown that heart block may be produced by asphyxia, and Florence Buchanan has pointed out that the phenomenon occurs physiologically in hibernating animals. There is, therefore, ample evidence to show that heart block is not necessarily due to recognized tissue changes in the node or bundle at the auriculoventricular junction.

It is seen that there is a fairly definite clinical picture of the heart in diphtheria. The pathologic findings are not so definite. The facts have been investigated, in general, through the study of (1) the myocardium; (2) the conducting system, and (3) the cardiac nerves. Investigators have described lesions in one or the other of these structures. A short summary will suffice to indicate their findings.

Aviragnet, Lutembacher and LeSoudier [21] have found two main types of myocardial lesions: (1) parenchymatous, and (2) interstitial and vascular. The parenchymatous changes consist in an alteration of almost all the fibers. They are swollen and vacuolated; the nuclei are pale, deformed, karyolytic, striations have disappeared, and there is more or less fragmentation. There are no fatty changes. The interstitial changes are chiefly, edema, slight leukocytic infiltration, and a small number of "infectious nodules" composed of mononuclears and fibroblasts.

Farr, in post-mortem examinations of a few cases, found foci of waxy degeneration in the bundle of His, in a case of heart block. In another case there was focal round cell infiltration of the sino-auricular

20. Rupe, W. A.: The Heart in Acute Infectious Diseases, Collective Abstract, Mod. Med. **2**:210, 1920.

21. Aviragnet, E. C.; Lutembacher, R., and LeSoudier: Le Coeur dans la Diphtérie, Arch. d. mal. du coeur **11**:241, 1918.

node and the bundle of His. The vagi were normal. In two other cases the bundle was uninvolved, although there was a widespread myocardial degeneration. In still another case there were fatty changes and a true myocarditis, but no involvement of the bundle.

Fleming and Kennedy [5] described the post-mortem findings in a case of complete heart block. The heart was distinctly dilated and rather pale. A section through the auriculoventricular node and first part of the auriculoventricular bundle showed an acute inflammation with round cells, congested capillaries, a few large mononuclears and some polymorphonuclears. Similar inflammatory foci with congestion of the capillaries were found in the auricle bordering on the nodal tissues. The ventricular muscle showed evidence of an interstitial myocarditis. Briefly, the heart was the seat of a myocarditis and the auriculoventricular node and first part of the auriculoventricular bundle were involved in the inflammatory process. Both vagi were examined and found normal.

Hecht describes the anatomic changes in the heart as consisting of fatty degeneration, cloudy swelling, and interstitial round cell infiltration.

Hume and Clegg stained the auricular muscle with Sudan III and demonstrated an excess of fat, but the amount was not comparable to that in the ventricular muscle. Occasional small hemorrhages were seen. The ventricular wall contained a very large quantity of fat which was diffused throughout the muscle of both ventricles; nearly every fiber contained some droplets. In some fibers it occurred as fine granules; in others as large round globules. The smallest capillaries were dilated and engorged. The sino-auricular node showed increased vascularity and actual hemorrhage, and increased infiltration with lymphocytic cells. The auriculoventricular node and bundle of His showed engorged and dilated capillaries.

Nuzum [22] described an eosinophylous myocarditis in seven out of twenty-nine cases of diphtheria, which was not present in many cases of death from various acute infectious diseases.

Osler [13] described fatty degeneration in a majority of cases, pointing out that it may precede the more advanced degeneration in which the sarcous elements become swollen and converted into hyaline masses.

In Price and McKenzie's [7] case of auricular fibrillation and heart block in diphtheria, the heart grossly was soft and flabby, the ventricular chambers greatly distended. The histologic findings may be summarized as extreme degeneration, cellular infiltration of the cardiac muscle, especially in the ventricle, and in scattered foci of unequal size dis-

22. Nuzum, F.: Eosinophylous Myocarditis in Diphtheria, J. A. M. A. **73**: 1925 (Dec. 20) 1919.

tributed largely in the course of the vessels. These changes did not involve the sino-auricular node or bundle. There was no evidence of change in the nerve trunks and ganglion masses in the posterior walls of the auricle. They comment that no definite anatomical lesions have been found to be characteristic of auricular fibrillation.

Tanaka [9] reports that of fifteen subjects dying in cardiac syncope, fatty degeneration was present in fourteen. Interstitial infiltration with polynuclear and mononuclear leukocytes and eosinophils were observed in six of these cases. Fragmentation of the muscle fibers was observed only once. Heilbecker found similar cellular changes. D'Espine and Mallet described parietal thrombi in both auricular and ventricular walls, as well as a parenchymatous myocarditis in a case of diphtheria dying in cardiac syncope. Hume says that the nearest approach to a lesion of the auriculoventricular node or bundle found at necropsy was a slight increase in vascularity.

Councilman, Mallory and Pearce [23] give an excellent summary of the pathologic changes in the heart associated with diphtheria. The following is a summary of their report:

1. Degeneration of the myocardium is one of the most common conditions found in diphtheria.

2. The simplest form of this is fatty degeneration, which is found in the majority of cases. This varies in extent, at times affecting the myocardium generally, at times occurring in foci. It may appear as fine granules at the junction of the transverse and longitudinal striations, or large globules involving the greater part of the substance of the muscle cell. Fatty degeneration accompanies and seems to precede the more advanced forms of degeneration which lead to the complete destruction of the muscle. Sarcous elements become broken and change to hyaline, or large vacuoles are formed.

3. Fragmentation and fractional degeneration of the muscle are often found, but segmentation or separation of the cells along the lines of junction does not take place, or is very limited.

4. Simple fatty degeneration is found in the severe cases of short duration, the more extensive degeneration in more prolonged cases.

5. Degeneration may be so extensive as to account fully for the impairment of the heart action.

6. Acute interstitial lesions of two sorts are found:

(*a*) Focal collections of plasma and lymphoid cells in the tissue, accompanied by degeneration of the myocardium but not dependent on it.

(*b*) Interstitial changes consisting of proliferation of the cells of the tissue, which is secondary to the degeneration of the muscle. This may lead to extensive formation of connective tissue, and some of the cases of fibrous myocarditis may be due to this.

7. Thrombosis is not an uncommon condition, due to primary necrosis of the endocardium. Lesions of the vessels of the heart play but little part.

---

23. Councilman, W. T.; Mallory, F. B., and Pearce, R. M.: A Study of the Bacteriology and Pathology of 220 Fatal Cases of Diphtheria, J. Boston Soc. M. Sc. **1**:137, 1901.

Our own studies on the heart in diphtheria have consisted chiefly of (1) investigations of human cases coming to necropsy, and (2) observations on the effects of diphtheria toxin on the guinea-pig heart.

The human material studied was obtained from cases occurring in the New Haven Hospital from 1917 to 1922, inclusive. During these years there were approximately 425 cases of diphtheria in the hospital. Of the total number, sixty-five patients, or 15 per cent., died. Of the sixty-five fatalities, nineteen, or 29 per cent., came to necropsy, and it is from these that the data to be described were obtained. The case histories and pathologic sections were critically studied in each case.

The diagnosis of myocarditis as a cause of death was made clinically in three cases out of seventeen, while endocarditis as an anatomic diagnosis was made in only two cases. With one exception, the patients were all children, the average age being 3.6 years. On this point it may be said that Richardson finds the greatest number of deaths under 5 years, while Osler states that in New York, from 1891 to 1900, 80.9 per cent. of the deaths were in children under 5 years.

The average day of illness on admission of these seventeen patients was 5.8 days. Antitoxin was given in fifteen out of the seventeen cases in amounts varying from 4,000 to 50,000 units, in but a single case given before the day of admission. The average duration of illness was 10.8 days. It may be recalled that Osler says that heart symptoms are more common in the second or third week of the disease, and that fatal dilatation of the heart may occur as late as the sixth or seventh week. Farr thinks that cardiovascular symptoms in diphtheria occur principally at two periods (1) early, at the end of the first fortnight when the local lesions (membrane, etc.) are characteristic and just coincident or preceding the palsies, and (2) late, i. e., in the midst of convalescence (third or fourth week or later).

The physical examination of the heart in these seventeen cases revealed enlargement of the heart to percussion in four cases. The heart sounds were reported feeble in four cases. Three instances of arrhythmia were observed, a sinus irregularity not respiratory (in a patient of 42), an atypical gallop rhythm, and a typical gallop rhythm.

The gross pathologic finding showed dilatation of the right side of the heart in seven cases. This was marked, however, in only one instance. In sixteen of the seventeen cases a terminal bronchopneumonia was found. In six cases there was a fibrinous pleurisy, and in three, an acute cervical lymphadenitis.

The heart was examined histologically in all cases. From one to five sections from each left ventricle was practically the only material available in the first fourteen cases. Very few pathologic changes were found. The analysis of the necropsy findings shows that: (1)

in no case was there a definite myocarditis, that is, a true inflammatory lesion; (2) the pathologic findings were more or less cloudy swelling and fat accumulation, and slight increase in the number of interstitial cells, being mostly mononuclears.

In view of the fact that there was so little abnormality found, it was thought advisable to study, by way of comparison, the heart in a group of cases in which death occurred from some infection other than diphtheria. There were selected for this purpose three cases of cerebrospinal meningitis, one case of scarlet fever, four cases of bronchopneumonia and six cases of measles.

One section from the left ventricle of each case was the only material available. In three cerebrospinal meningitis cases the heart was practically normal. In the bronchopneumonia group there was one case in which there was a definite focal myocarditis with mononuclear and polymorphonuclear infiltration, and another case showing an increased number of mononuclear cells in the cardiac septa. The measles group showed only a slight increase in cellular reaction in the interstitial tissue of the heart. In brief it was found that the myocardium in infections other than diphtheria shows a slight increase in cellular elements in the interstitial tissue, with, however, no definite acute inflammatory reaction.

For further investigation the heart was studied in a third group of cases in which the organ was presumably normal. All were noninfectious cases. These were difficult to obtain because in children the great majority of deaths are due to infection. One section of the heart from each of these cases was studied. The tissue in some showed a slight increase in the number of interstitial cells; no instance of a definite inflammatory reaction was encountered.

A comparison of the three groups of cases studied, diphtheria, other infections, and "normal" heart, respectively, shows that the differences are quantitative rather than qualitative. There is no sharp line between the normal and the abnormal; and no clear cut difference can be detected in the hearts from diphtheria cases and those from other types of infections. In other words, the pathologic study of this material indicates that the lesion in the myocardium in diphtheria is not specific.

The first fourteen cases cited above furnished material which was somewhat incomplete as respects both clinical and pathologic data. Opportunity, however, was afforded to make a more intensive study of three additional cases which came to necropsy during the last six months.

One case is an excellent example of a myocardial death in diphtheria, diagnosed as acute myocarditis clinically and showing a striking myocarditis pathologically. The following is a brief summary of the case.

REPORT OF CASE

T. G. (Hospital No. 3891), aged 7 years, was admitted Dec. 20, 1921.

Complaint.—Diphtheria.

Family History.—Family living and well. Mother, living and well. Pregnancies, four normal; no miscarriages. No history of tuberculosis.

Past History.—Full term, normal delivery; breast fed; development normal; always had a deformed chest. Acute infections: pertussis, uncomplicated. Had vomiting attacks about every four months; mother gives castor oil and attacks subside.

Present Illness.—Onset seven days ago (Dec. 14, 1921). Vomiting attack; mother gave castor oil which did not help very much. Vomited next day also, especially after meals. Two days later (December 16) the child complained of sore throat and mother noticed that night a swelling in the neck. A doctor was called the next day; he made a diagnosis of diphtheria and gave 10,000 units of antitoxin. Glandular swelling did not subside although the child improved. Cultures were positive.

Physical Examination.—GENERAL: Child not prostrated; face flushed; lips red; breathing quiet. Head: normal in size and shape; depression in region of anterior fontanel. Pupils equal and react to light and accommodation, extra-ocular movements normal; conjunctivae of good color. Nose: Evidence of epistaxis, no discharge. Ears: No discharge; drums normal. Mouth: Teeth carious and incisors notched. Tongue: Clear; papillae not prominent; red at tip; buccal mucosae clear; pharynx, red; grayish white membrane over both tonsils and extending up on soft palate to side of uvula. Glands: At angles of jaws glands are swollen and tender; axillaries palpable; right epitrochlear gland very small and just palpable. Chest: Exraordinary appearance; left side of chest is very much deformed and thinned so that the heart is visible and palpable; pulsating very superficially under a thin wall; sternum is elevated and forms a crest. Lungs: Clear. Heart: Apex not made out and borders not at all clear; visible pulsation seen at base of heart; no thrill palpable; loud systolic bruit heard, localized at base; rate not rapid; sounds of good quality and regular. Abdomen: Spleen not felt, liver at costal margin; no masses or tenderness. Genitals: Left testicle undescended. Reflexes: Active and equal.

Diagnosis.—Diphtheria, pharyngeal; depression of chest.

Course.—By December 23, the throat was only slightly reddened. Tonsils enlarged but exudate all gone. Lymph nodes at angle of jaw still swollen and tender.

December 24, heart showed gallop rhythm and was at times irregular. Liver, tender, one and one-half finger breadths below costal margin. Lungs, clear. Later on same day child became worse. Complained of pain in the abdomen. Abdomen rigid, especially in right upper quadrant. Heart weak, gallop rhythm; pulse feeble. Face has anxious expression. Night of December 24, rapid breathing, lips cyanotic, face pale, liver edge at level of umbilicus. Pulse became impalpable. Heart irregular and of feeble quality. Patient died suddenly after raising his head from the pillow.

Clinical Diagnosis.—Pharyngitis, diphtheritic; myocarditis, acute toxic.

Temperature: December 20, 99.8 F.; December 21, 101.0 F.; December 24, 98.6 F.

Respiration: 20.

Pulse: December 20, 110; December 23, 80; December 24, 96.

Hemoglobin, 75 per cent.; red blood cells, 4,820,000; white blood cells, 14,400, December 20; 53,000 December 24.

Urine: albumin, 0.5 gm. per liter December 24 (slightest possible trace December 20).

Pathologic Findings.—(Necropsy 579.) Gross: Body length, 154 cm.; weight, 16 kilos; weight of heart, 120 gm.

A small milky patch is found on the anterior surface of the right ventricle. In the right auricle is an inelastic friable blood clot attached to the musculi pectinati. The right ventricle is dilated. The left ventricular cavity is slightly dilated. The heart muscle is homogeneous pale brown in color.

Microscopic: Blocks were taken from the right auricle, interauricular septum, left ventricle, middle, left ventricle, apex, interventricular septum, and right ventricle. (Paraffin sections were stained with hematoxylin and eosin; frozen sections were stained with Sudan III for fat.)

Hematoxylin-Eosin Sections: There is a striking change in the heart muscle. (1) The muscle fibers appear degenerated in areas. The protoplasm is homogeneous or is absent, mere strands of tissue being left. There is actual necrosis of muscle fibers in which the nuclei have disappeared. Many of the muscle bands are widely separated. (2) There is a marked mononuclear cell infiltration, the cells occurring in foci. Among them may be seen eosinophils. Some of these foci include over a hundred cells and separate the muscle fibers widely. (3) There are some red blood cells free among the fibers. (4) Strands of fibroblasts are seen between the muscle fibers.

The changes were most clear-cut in the left ventricle but were observed in all parts of the heart studied. The interauricular septum showed in addition several areas of hemorrhage. The right auricle contained a thrombus. The accompanying illustration shows these changes very well.

Fat Stains: Throughout the whole there is a marked fatty infiltration. The fat is focal in distribution and these foci are scattered throughout the myocardium. The droplets or granules fill the cytoplasm of the muscle elements. Each focus consists of a number of muscle fibers. The left side of the heart is more affected than the right and the interventricular septum more than the outer walls.

Case 2.—H. A. (Hospital No. 1787), aged 2½ years, entered the hospital Aug. 18, 1921.

Complaint.—"Can't breathe."

Family History.—Father has cough, possibly tuberculous; father's sister is in a tuberculosis sanitarium; mother, living and well. Pregnancies: five normal, no miscarriages.

Past History.—Full term, normal delivery; breast fed for three months. Screams out in sleep. Eyes filled with pus in morning. Attacks of croup. Has numerous earaches. No history of any acute infection. Has had intestinal worms for about five months.

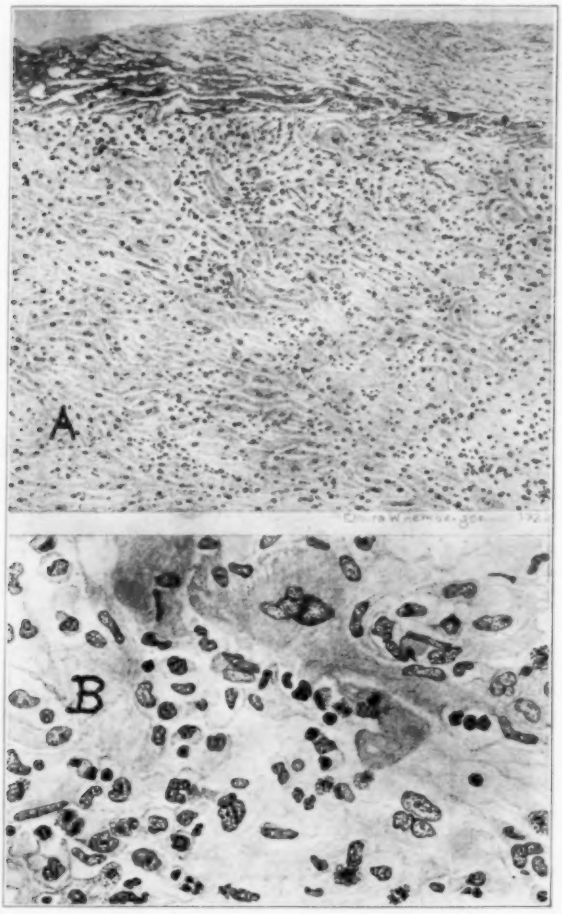
Present Illness.—The onset was twenty-four hours before admission. The patient awoke from an afternoon nap with difficulty in breathing. He became steadily worse and at night became very restless; had deep labored breathing; voice hoarse. Had fever and refused food. Became worse; doctor saw him the next afternoon and said he had diphtheria and sent him to the hospital.

Physical Examination.—General: The child presented a striking picture, with difficult breathing, anxious expression, face flushed with pallor about the mouth, body surface temperature considerably raised. Lymph Glands: Palpable at angle of jaw. Face: Some suggestion of facial weakness on right. Lips: Slightly cyanosed. Throat: Pharynx considerably injected; tonsils slightly enlarged and injected; post-pharyngeal wall injected; over lower pole of right tonsil is a small, white patch of exudate. Larynx: Voice is hoarse and barely audible; cough whistling and dry. Chest: Well formed and symmetrical; on inspiration there is a considerable retraction of the suprasternal notch, also of the interspaces, and the lower ribs and costal margins sink in at each inspiration; retraction of the xiphoid. Lungs: Respirations rapid and labored; coarse, moist râles, laryngeal in character, heard over whole

chest. Heart: Not enlarged; rate very rapid; heart sounds clear; no shocks, thrills or murmurs.

Diagnosis.—Diphtheria, laryngeal.

Course.—August 18: Intubation.
August 19: Breathing more difficult.
August 22: Whooping cough noted.



Acute myocarditis in a case of fatal diphtheria (Necropsy 579). *A*, low power. *B*, high power.

August 25: Laryngeal stridor.
August 27: Bronchopneumonia.
September 1: Suddenly became worse; pulse could not be counted; dyspnea; tracheotomy done; convulsions followed by death.

Final Diagnosis. — Laryngeal diphtheria; pertussis; bronchopneumonia; chronic ulcerative laryngitis; ascaris lumbricoides infection. Temperature, from 108 to 98.8 F. Respiration, from 24 to 88. Pulse, from 112 to 190 per minute. Hemoglobin, 85 per cent.; red blood cells, 4,950,000; white blood cells, 27,000.

Pathologic Findings. — (Necropsy 540.) Gross: Length, 82 cm.; weight, 11.3 kilos; weight of heart, 60 gm. The right auricle and ventricle are moderately dilated. The auricle contains an elastic clot.

Microscopic: Blocks were taken from the right auricle, interauricular septum, left ventricle, middle, left ventricle, apex, interventricular septum, and right ventricle. (Paraffin sections were stained with hematoxylin and eosin; frozen sections were stained with Sudan III for fat.)

Hematoxylin-Eosin Sections: The muscle fibers appear normal in all the sections studied. A few of the blood vessels are congested and there are several small foci of mononuclear infiltration. A few fibroblasts are seen here and there.

Fat Stains: There is practically no fat seen in any part of the heart muscle which was studied.

Case 3.—S. W. (Hospital No. 2409), aged 7½ years, entered July 27, 1921.

Complaint.—Difficulty in breathing.

Present Illness.—Onset one day before admission. Difficulty in breathing which grew worse. Had slight cough.

Physical Examination. — Marked retraction of ribs with inspiratory and expiratory difficulty. Marked drawing in of episternal notch during inspiration. Membrane on tonsils.

Course.—Difficulty in breathing with retraction continued in spite of intubation. He became cyanotic; heart slightly irregular. Tracheotomy done and cast of membrane 2.5 cm. long and 0.5 cm. in thickness was removed from the trachea. Obstruction below the tube became so marked that in spite of surgical treatment, respiration ceased.

Diagnosis. — Diphtheria, pharyngeal, laryngeal and tracheal; bronchopneumonia.

Temperature, from 100 to 104 F. Respiration, from 40 to 60 per minute. Pulse, 146 to 160 per minute. Hemoglobin, 80 per cent.; red blood cells 5,120,000; white blood cells, 18,000.

Pathologic Findings (Necropsy 516). — Gross: Length, 85 cm.; weight of body, 18.2 kilos; weight of heart, 60 gm.

There was very little subepicardial fat. The chambers of the heart showed slight dilatation. The endocardium was a homogeneous dark red.

Microscopic: Blocks were taken from the right auricle, interauricular septum, left ventricle, middle, left ventricle, apex, interventricular septum, and right ventricle.

Hematoxylin-Eosin Sections: The heart muscle fibers appear fairly normal throughout the sections. There are mononuclear cells scattered here and there but there are no definite foci of these cells.

Fat Stains: There is no fat present in any part of the heart studied.

Comment.—It will be seen that only the first of these three cases represents a typical acute clinical myocarditis of diphtheria, and that anatomically this finding is borne out by the presence of a striking acute myocarditis with characteristics as described above.

The second case did not show a myocardial change either clinically or anatomically.

The third case did not show a myocarditis clinically, but did show a mild myocardial involvement pathologically.

As the fat changes were so striking in the case of typical acute myocarditis, two relatively normal hearts of about the same age were stained for fat to study their content.

1. D. DeL., aged 3 years (Hospital No. 1478, Necropsy 518), died of fibrosarcoma of nasopharynx. The heart showed no fat in the muscle fibers.

2. E. O., aged 6 years (Hospital No. 3698, Necropsy 203), died of glioma of brain. The heart showed no fat in the muscle fibers.

In order to see if a myocarditis might be produced experimentally by diphtheria toxin, four guinea-pigs were injected with the toxin. The table shows dosage, length of life, etc.

SUMMARY OF DATA OF GUINEA-PIG EXPERIMENTS

Guinea-Pig No.	Weight in Gm.	First Date	Initial Dose*	Subsequent Dosage, Fourth day	Died	Lived, Days
1	425	7/26/21	1 c.c. of 1:300	1 c.c. of 1:150	7/30/21	9
2	425	7/26/21	1 c.c. of 1:300	1 c.c. of 1:150	8/ 1/21	7
3	400	7/26/21	0.5 c.c. of 1:300	1 c.c. of 1:150	8/ 2/21	8
4	400	7/26/21	0.5 c.c. of 1:300	1 c.c. of 1:150	8/ 6/21	12

* The diphtheria toxin was obtained from the New York City Board of Health; M. L. D. equals 1/600 c.c. for guinea-pig.

In order to simulate the gradual progressive action of diphtheria toxin in the human, it was decided not to inject immediately the M.L.D., but to give less at the first injection. This was done, and the first two guinea-pigs were injected intramuscularly with 1 c.c. of 1:300, and the second two with 0.5 of 1:300, respectively. As all the animals were living on the fourth day, they were each injected with 1 c.c. of 1:150 toxin.

The first animal died on the fifth day, the next on the seventh, and the last two on the eight and twelfth days, respectively.

The hearts in each case were studied pathologically, hematoxylin and eosin stains and Sudan III for fat being made. They showed:

Guinea-Pig 1.—Hematoxylin and Eosin Stain: The muscle fibers appear somewhat cooked. There are red cells scattered free between the muscle fibers usually appearing singly. There are a few young fibroblasts in strands seen between the muscle fibers. The heart is very vascular.

Sudan III: There is a marked deposit of fat in the muscle fibers. This is focal in nature, appearing especially around the nuclei and involving a large number of muscle fibers. The fat accumulation seems to be more marked in the inner portion of the heart wall, that is in the subendocardial zone.

Guinea-Pig 2.—Hematoxylin and Eosin Stain. The muscle fibers have a somewhat cooked appearance. There are a few foci of large mononuclear cells. The blood vessels of the myocardium are not so conspicuous as in Guinea-Pig 1.

Sudan III: The fatty infiltration is marked. There are large foci of fat throughout the heart and many fibers are involved. In this case the fat appears to be nearer the outer than the inner wall of the heart.

Guinea-Pig 3.—Hematoxylin and Eosin Stain. The muscle fibers appear to be somewhat thinned out. No mononuclear cell accumulations are seen.

Sudan III: There is practically no fat seen in the muscle fibers.

Guinea-Pig 4.—Hematoxylin and Eosin Stain. The heart muscle appears somewhat cooked. Capillaries are conspicuously congested.

Sudan III: There is practically no fatty infiltration of the heart muscle fibers.

A study of the hearts of two healthy guinea-pigs was made to determine the character of the normal myocardium and its fat content.

Normal A.—Hematoxylin and Eosin Stain: The heart muscle fibers appear normal. The blood vessels are numerous. There are a few areas of mononuclear infiltration, especially perivascular. There are also some large mononuclear cells between the muscle fibers.

Sudan III: There is no fatty infiltration.

Normal B.—Hematoxylin and Eosin Stain: The heart muscle appears normal. The blood vessels are numerous. There are a few areas of mononuclear infiltration around the blood vessels and between the muscle fibers. The heart is essentially like that of Normal A.

Sudan III: There is no fatty infiltration.

It will be seen that the first two guinea-pigs which received the larger initial dose and which died first, showed marked fatty infiltration. The distribution and character of the fat corresponds exactly to that found in the human heart in the first case cited, although the myocardium does not show the focal necrosis of the fibers and cellular infiltration so marked in that case.

These experiments, we believe, are sufficient to show that while diphtheria toxin does damage the heart as evidenced by a pronounced focal fat accumulation in the fibers, it does not, even in fatal doses resulting in delayed deaths, bring about a true myocarditis. In this respect the findings agree with those in the majority of fatal cases of human diphtheria.

SUMMARY

1. Clinical evidence indicates that a large proportion of the fatalities in diphtheria are due to circulatory failure (Herztod).

2. Death in these cases is probably referable to injury to the myocardium itself or its conducting system, although experimental evidence (MacCallum) points to a serious disturbance in the peripheral vasomotor system.

3. A definite inflammatory reaction (true myocarditis) is only exceptionally demonstrable at necropsy (one out of nineteen cases in the present series). Fat accumulation and cloudy swelling are very regularly seen, but are not more pronounced than in other acute infectious diseases such as scarlet fever, measles, and pneumonia. In other words, no specific myocardial lesion has been demonstrated.

4. The injection of lethal or sublethal doses of diphtheria toxin in guinea-pigs produces degenerative changes in the heart muscle similar to those seen in the human heart in fatal diphtheria, but such injections do not induce a true inflammatory reaction.[24]

---

24. The following references also bear on this subject:

Abbott, A. C., and Ghriskey, A. A.: A Contribution to the Pathology of Experimental Diphtheria, with Special Reference to the Appearance of Secondary Foci in the Internal Organs, Bull. Johns Hopkins Hosp. **4**:29, 1893.

Cronk, H. L.: The Heart in Acute Febrile Diseases, Practitioner **104**:102, 1920.

Figdor, P.: Pericarditis in Diphtheria, Proc. Roy. Soc. Med. **8**: Sect. Dis. Child., p. 89, 1913.

Flexner, S., and Anderson, H. P.: Results of Intracranial Inoculation of the Bacillus Diphtheriae in Rabbits, Bull. Johns Hopkins Hosp. **9**:72, 1898.

Goodpasture, E. W.: Myocardial Necrosis in Hyperthyroidism, J. A. M. A. **76**:1545 (June 4) 1921.

Gordon, A. K.: The Clinical Diagnosis of Diphtheria, Lancet **1**:1239, 1920.

Harding, M. E.: The Circulatory Failure of Diphtheria, London, 1920.

Nuttall, G. H. F., and Graham-Smith, G. S.: The Bacteriology of Diphtheria, Including Sections on the History, Epidemiology, and Pathology of the Disease, the Mortality Caused by It, the Toxins and Antitoxins and the Serum Disease, Cambridge, 1913.

Park, W. H.: The Rôle of Animal Experimentation in the Discoveries Leading to Our Present Knowledge of the Etiology, Prevention and Cure of Diphtheria, J. A. M. A. **58**:453 (March 10) 1912.

Pettit, A.: Action de la toxine diphtérique sur le rat, Ann. de l'Inst. Pasteur **28**:663, 1914.

Place, E. H.: Diphtheria, Oxford Med. **5**:125, 1921.

Sperk, B.: Ein Fall von Adams-Stokeschen Syndrom nach Diphtherie, Wien. klin. Wchnschr. **26**:1094, 1913.

Welch, W. H., and Flexner, S.: The Histological Changes in Experimental Diphtheria, Bull. Johns Hopkins Hosp. **2**:107, 1891.

Welch, W. H., and Flexner, S.: The Histological Lesions Produced by the Tox-Albumin of Diphtheria, Bull. Johns Hopkins Hosp. **3**:20, 1892.

Wright, J. H.: Studies in the Pathology of Diphtheria, Boston M. & S. J. **131**:329, 1894.

THE PRODUCTION OF ATHEROSCLEROSIS IN RABBITS BY FEEDING DIETS RICH IN MEAT*

L. H. NEWBURGH, M.D., AND SARAH CLARKSON, M.S.
ANN ARBOR, MICH.

In an earlier communication, Newburgh and Squier [1] reported the occurrence of atherosclerosis in two small groups of rabbits (eleven in all) that had been fed high protein diets. While the evidence at hand indicated that the vascular lesions had been caused by the high protein diets, such a conclusion was not safe because it was felt that the experiments were too few to permit a generalization.

This paper deals with the further investigation of the effect of high protein diets on the arteries of rabbits; but the presentation of the new material will be preceded by a definition of the term "atherosclerosis," and by a discussion of the work of earlier students of experimental atherosclerosis.

DEFINITION

The causes of human atherosclerosis have been sought for, by means of animal experimentation, by a large number of investigators. They have attempted to injure the vessels by the use of a great many different procedures, and a variety of vascular lesions have been reported. But much of the work, and more especially the earlier studies, have not contributed to the understanding of the problem since so often the lesion obtained in the experimental animals was in no sense the analogue of the human disease. The investigator must, by the use of the agent which he suspects of being a cause of human atherosclerosis, produce in animals a picture which at least closely resembles what is found in the human being before he can with justification claim that this agent may be a cause of human atherosclerosis.

The term atherosclerosis as used in dealing with the diseases of arteries occurring in human beings, is intended to designate a primary lesion of the intima, and to exclude lesions of the intima which result from disease of the media or vasa vasorum. The process presents itself to the naked eye, in its most characteristic stage, in the form of discrete or confluent, slightly raised, pale yellow, opaque plaques and streaks. This earlier form of the lesion may later be altered by softening of the contents of the nodule and the loss by ulceration of the caseous material thus formed. The deposition of calcium salts in

* From the Department of Internal Medicine, Medical School University of Michigan.

1. Newburgh and Squier: Arch. Int. Med. **26**:38 (July) 1920.

large amounts converts the base of the ulcer into a hard but brittle depression. But these well recognized gross appearances are not always sufficient to permit the observer to distinguish between primary atherosclerosis and other vascular lesions which affect the intima secondarily —in particular syphilis of the aorta; and in experimental work, the microscopic features should be used in reaching a decision. The histologic picture presented by this primary disease of the intima depends on the stage at which it is seen.

The initial lesion consists essentially of a necrosis or fatty degenerative change in the endothelium or the fibroreticular cells of the intima. The yellow elastic fibers are separated and pushed apart by the swollen fatty cells. With increasing injury the elastic fibers undergo a granular degeneration and break up into a granular detritus.

Following the stage of primary injury, a regenerative compensatory fibrosis occurs with the production of new elastic fibers. The growth of new elastic fibers is often marked and usually occurs chiefly on the lumen side of the lesion so that the degenerated area comes to appear to be located nearer to the media than to the lumen.

A secondary degeneration of this sclerotic area occurs and may result in rupture of the atheromatous patch into the lumen of the vessel, forming the so-called atheromatous ulcer. In advancing lesions there is a progressive fatty degenerative change involving not only the intima but extending into the interlamellar fibroreticulum of the media. At any time calcification may be an added factor. The two processes, the degenerative and the regenerative-compensatory, go hand in hand and in old progressive cases may lead to the most marked changes of the vessel wall extending even to the adventitia.

When one wishes to determine whether an experimental vascular lesion is atherosclerotic in type, it is always desirable to examine the earlier stages of the lesion. In primary disease of the intima it will be noted that this coat has undergone great thickening and that its increased width is chiefly due to the presence in it of large cells loaded with fat imbedded in a hyperplastic elastic tissue. The coexistence of the enormous amounts of fat and the hyperplasia of the elastic tissue stamp the lesion in question as a true primary disease of intima—an atherosclerosis.

LITERATURE

Failure to use these generally accepted criteria as a basis for determining whether any given lesion could properly be considered atherosclerotic has given rise to much confusion. Thus Saltykow,[2] in his review of the literature published in 1908, was compelled to point out

2. Saltykow: Centralbl. f. Allg. Path. **19**:321, 1908.

that very little of the experimental work up to that date bore on the problem of the cause of human atherosclerosis since the lesions obtained in the animals did not resemble the human type. The use of mechanical methods, the injury of nerves, the injection of alcohol, lead and epinephrin did not cause vascular disease which resembled human atherosclerosis.

Epinephrin.—But the experiments with epinephrin, even though they failed to produce primary intimal disease, must, nevertheless, be given serious consideration since the injection of epinephrin causes a type of vascular lesion which is frequently seen in man and which by a few writers is included under the heading of atherosclerosis. Mönckeberg [3] showed that this lesion, characterized by necrosis and calcification of the media, is a pathologic entity, originating in and often restricted to the media, and clearly separable from the primary intimal disease. Mönckeberg pointed out that the calcification of the media was first recognized by Virchow and separated by him from "atheroma" (disease of the intima). Some writers have expressed the belief that calcification of the media and atherosclerosis were both responses to a common etiologic factor. In support of this belief, they point out that the peripheral vessels in which calcification is most often found possess thick muscular walls, whereas, the aorta and other central arteries have relatively little muscle in their walls. They assume that these differences are sufficient to cause the reaction to an insult to take place in the media when the vessel is thick walled and in the intima when it is thin walled. But Mönckeberg showed that this hypothesis did not conform to the facts, for primary intimal changes are frequently seen in vessels of the muscular type; medial calcification occurs in the central arteries; often both lesions occur in the same artery. He concluded that medial calcification cannot be the anatomic equivalent of the intimal disease of the aorta. It is important to make this distinction between true atherosclerosis and calcification of the media since it is only on this basis that a proper evaluation of the epinephrin work can be made. The intravenous injection of epinephrin into rabbits produces lesions which are identical with the Mönckeberg type of human vascular disease. Josué,[4] who was the first to study the effect of epinephrin on the vessel wall, announced in 1903 that he had obtained "atheroma" of the rabbit's aorta. His statement, however, was based only on the macroscopic appearance of the vessel. Subsequently he agreed with later workers that the lesion was primarily medial. The radical differences between the epinephrin changes in the rabbit's aorta and human atherosclerosis are well sum-

3. Mänckeberg: Arch. f. path. Anat. **216**:408, 1914.
4. Josué: cited by Saltykow.[2]

marized by Saltykow, as follows: "In man the disease is localized in the intima; in the rabbit in the media. In man the lesion shows a high grade fatty degeneration; in the rabbit little or no fat is seen. In man calcification occurs as the end stage of the degenerative process; in the rabbit it appears at the beginning of the disease." But in spite of these basic differences, some experimentors have nevertheless maintained that the epinephrin lesions of rabbits were the analogue of human atherosclerosis, on the ground that epinephrin attacks the media in the rabbit's aorta because the latter is thick walled, whereas the thinness of the media in the human aorta forces the lesion into the intima. This argument is very similar to the one used by writers on human pathology who tried to show that medial calcification of the peripheral arteries is the anatomic equivalent of true atherosclerosis. The unsoundness of this argument was, as already noted, shown by Mönckeberg. Such reasoning becomes quite untenable in the light of two facts: (1) widespread and advanced disease of the intima of the rabbit's aorta, the counterpart grossly and microscopically of the human disease of the intima, may be produced by suitable methods. Saltykow [5] and Klotz [6] have produced atherosclerosis in rabbits by injection of bacteria. Ignatowski,[7] Steinbiss [8] and we have obtained the same type of intimal disease in the rabbit by feeding abnormal diets of various sorts.[2] Klotz [9] has shown that epinephrin has a selective action on muscle. It causes necrosis of muscle whenever it comes in contact with it in sufficient concentration, be it skeletal muscle, heart muscle or the smooth muscle of the media.

Epinephrin then, attacks the media of the rabbit's aorta for one reason only, namely, because the media contains muscle cells. The supposition that epinephrin produces medial calcification in the rabbit because atherosclerosis in the rabbit is by the nature of things, as is implied by some writers, a primary disease of the media, is quite untenable, since, as just pointed out, primary intimal disease may be produced in the rabbit by the use of proper agents. Epinephrin fails to produce primary intimal lesions because epinephrin has no affinity for the cells of the intima. If the arteries of man are ever damaged by it, the lesions must occur in the media and produce the Mönckeberg calcification of the media.

Bacteria.—Saltykow [2] was accordingly quite justified in his statement in 1908 that the only method which caused, in the rabbit, vascular

5. Saltykow: Beitr. z. path. Anat. **43**:147, 1908.
6. Klotz: J. Exper. M. **12**:707, 1910.
7. Ignatowski: Arch. f. path. Anat. **198**:248, 1909.
8. Steinbiss: Arch. f. path. Anat. **212**:152, 1913.
9. Klotz: Brit. M. Jour. **2**:1767, 1906.

lesions that could be considered the analogue of human atherosclerosis was the injection of bacteria. Gilbert and Lyons (quoted by Saltykow), as early as 1889, occasionally obtained intimal lesions following the intravenous injection of an organism recovered from a patient with endocarditis. Subsequent workers reported a few positive results but more often failed to produce a lesion which resembled the human type sufficiently to justify its being called atherosclerosis. Finally Klotz,[9] in 1906, published experiments which showed conclusively that intimal lesions of the atherosclerotic type could be produced regularly by the intravenous injection of bacteria (typhoid bacillus and streptococcus) into rabbits. Saltykow[5] in 1908 reported the same type of intimal lesion following the injection of different strains of the staphylococcus.

These two groups of experiments seemed to have established beyond reasonable doubt the idea long held by students of the subject, that atherosclerosis is often caused by infection. But unsuccessful attempts of later workers to confirm these experiments reopened the whole discussion and led Saltykow to repeat his work on a more elaborate scale.

Abnormal Diets.—Before taking up Saltykow's second paper, it is necessary to review some of the work of a group of investigators who had shown that abnormal diets could cause atherosclerosis, since Saltykow's later experiments incorporated the ideas emanating from the work with these diets. Ignatowski[7] fed rabbits varying amounts of meat, eggs and milk and obtained lesions of several organs, including the aorta. He stated that "all rabbits fed animal protein showed aortic lesions of the same type. They consist of confluent and single plaques, yellow, round or irregular." Stuckey[10] devised experiments to discover what portion of the animal diet was responsible for the lesion. For this purpose he fed four series of rabbits as follows: (1) cow's milk plus egg albumin; (2) cow's milk plus meat juice; (3) cow's milk plus egg yolk; (4) cow's milk to which egg albumin, egg yolk and meat juice was added. Only the animals of the third and fourth series that had received the egg yolk showed well marked vascular lesions. These data led him to conclude that the egg yolk was responsible for the lesion and that the protein contained in the animal diets had little to do with the vascular disease. Chalatow[11] had, in the meantime, studied the effect on the liver of feeding egg yolk, and had found a marked infiltration of liver cells by fatlike substances whose physical characters showed them to be rich in cholesterol. Anitschkow and Chalatow[11] then fed cholesterol dissolved in hot sunflower seed oil to rabbits. After a few weeks they saw an infiltration of the liver parenchyma with cholesterol.

10. Stuckey: Centralbl. f. Allg. Path. **12**:379, 1911; **13**:910, 1912.
11. Chalatow: Centralbl. f. Allg. Path. **24**:1, 1913.

The same material was found in the aorta, spleen and all other organs examined. They believed that these experiments explained the results noted by earlier investigators who had fed abnormal diets; and that all such diets had produced lesions because they contained an excess of cholesterol. Wacker [12] in the same year called attention to the occurrence of atherosclerosis in rabbits to whom cholesterol mixed with oats had been fed. The animals ingested about 1.5 gm. cholesterol daily. The cholesterol values in the blood rose slowly, and the increase in some instances was striking. The report deals with eight rabbits, five of which were fed for more than 120 days. Four of these showed aortic atherosclerotic lesions at the necropsy—widespread in three instances, but minute in one animal that had eaten the cholesterol 160 days. The rabbit that ate the cholesterol for the greatest number of days had the highest blood cholesterol value but a normal aorta.

Wacker himself pointed out that the lesions, in degree and extent, were not parallel to the amount of cholesterol fed nor to the duration of the experiment.

Saltykow [13] was convinced that the cholesterol contained in the animal diets had caused the vascular disease and pointed out that the aortic lesions found in the rabbits that had been injected with staphylococci by him and which had formed the basis of his report in 1908 might have been in part or wholly due to the diet, since his animals had received a considerable amount of milk. In order to decide this question, he injected various strains of staphylococci into a group of rabbits whose diet contained 100 c.c. of milk daily. A second group of rabbits received no treatment other than the addition to the diet of 400 c.c. of milk daily. He found that milk feeding caused as great changes in the aorta as the combination of milk feeding and the injection of bacteria. Furthermore, the lesions obtained in milk fed rabbits that had been injected with staphylococci were identical with the lesions seen in the animals that had received milk only. These lesions were indistinguishable from those produced by cholesterol. Even though milk contains much less cholesterol than egg yolk, Saltykow's calculations showed that the total amount of cholesterol eaten by his milk fed rabbits was greater than the amount ingested by rabbits made atherosclerotic by being fed egg yolk and this led him to conclude that the chief etiologic factor in his experiments was the milk feeding but that the staphylococci had a contributory effect on the formation of the lesion. In so doing, he assumed that often repeated small doses of cholesterol would have the same ultimate effect as fewer large doses. But such

12. Wacker: Arch. f. Exper. Path. u. Pharmakol. **74**:422, 1913.
13. Saltykow: Beitr. z. path. Anat. **57**:415, 1913-1914.

an assumption is at variance with the pharmacologic principle that concentration is one of the prime factors in determining the effect which any substance will have on cells.

In the absence of an actual experimental demonstration that the small amount of cholesterol present in 100 c.c. of milk is capable of causing vascular disease, one is surprised to find that Saltykow did not adhere to his original contention, namely, that in the rabbits receiving injections of bacteria and 100 c.c. milk daily, the aortic disease was caused by the bacteria.

The vascular disease found in the rabbits that were fed 400 c.c. milk daily was presumably caused by some ingredient in the milk. But not necessarily by the cholesterol since the daily dose of it was still far below that contained in one egg. On the other hand, 400 c.c. milk contains approximately 15 gm. protein, an amount greatly in excess of that habitually eaten by a rabbit. Subsequent work has shown that high protein diets will cause atherosclerosis in the rabbit. Hence, one is not required by the data at hand to attribute the atherosclerosis to the cholesterol in the milk.

The investigation of the effect of abnormal diets upon the arteries was continued by Steinbiss.[8] He fed a diet made of dried powdered horse liver (freed of fat) mixed with moistened bread. The aorta of rabbits presented small raised plaques as early as two weeks from the beginning of the experiment. Microscopic examination showed these lesions to be medial and identical with the epinephrin injury. Even in the most advanced cases, the intima as a rule, was normal and never showed more than trifling changes, which when present were not those of human intimal disease. In some animals which were fed the longest time, the aorta was normal but the media of the peripheral vessels was extensively involved. The myocardium and skeletal muscle showed the same type of lesion as the media. Saltykow[12] repeatedly found medial lesions of this same type in treated and in fresh animals and believed that these necroses of the media were spontaneous. Steinbiss' experiments with this diet were so few and relatively of such short duration that they, taken by themselves, are not of much value. The paucity of data was caused by the very high mortality of the animals. But this difficulty was overcome by the occasional addition of a little carrot or other green food to the liver-bread mixture. The mortality was thereby greatly reduced and the condition of the animals improved in every way. A group of rabbits kept on this modified diet from four to six months showed no vascular lesions. It is difficult to believe that the addition of a very small amount of green food to the liver-bread diet would prevent the occurrence of the medial lesions over a period of six months, if such lesions could, in fact, be

caused by the ingestion of liver without carrots for only two weeks. Of far more importance are the data which Steinbiss obtained when he fed this same diet, made up of dried liver, bread and greens, to ten rabbits for nine months. The aortas of all of these ten animals showed well marked atherosclerosis of the human type.

The experiments of Ignatowsky, Saltykow and Steinbiss must be accepted as proof that diets containing large amounts of animal food will cause atherosclerosis in the rabbit. Ignatowsky, who used meat, milk and eggs in varying combinations, attributed the occurrence of the vascular disease in his animals to the presence of animal protein in the diet. This view, however, was not accepted by Stuckey, whose investigations led him to conclude that the lipoids and not the protein must be held accountable for the vascular injury. Then followed the work with cholesterol, which was generally accepted as a satisfactory demonstration that the cholesterol in the diet was the cause of the vascular lesions reported by Ignatowsky, Saltykow and others who had fed animal diets to rabbits.

But such a conclusion was not warranted by the evidence at hand for Ignatowsky's diets were not only high in cholesterol but also in protein; and even though Stuckey, Chalatow and Anitschkow had shown that diets high in cholesterol would cause extensive atherosclerotic lesions, they had not shown that diets high in protein but poor in cholesterol would not cause similar vascular lesions.

Knack[14] shortly thereafter questioned whether a simple hypercholesterinemia could cause atherosclerosis, suspecting that some other agent was always responsible for the primary lesion. He pointed out that the animal diets used by his predecessors were not only high in cholesterol but were also abnormal for rabbits in other respects and might thus give rise to metabolic disturbances which had caused the primary vascular injury attributed to the hypercholesterinemia. Such criticism, however, did not apply to the feeding experiments of Chalatow and Anitschkow since they used only oats, hay and greens to which cholesterol dissolved in hot sunflower seed oil was added. Knack wondered whether products of this oil had injured the intima.

Knack did not discuss the work of Wacker who had fed cholesterol and oats. But he could properly have insisted that a diet restricted to oats is in itself abnormal, and when long continued leads to metabolic disaster. Here, again, the primary vascular injury could conceivably have been caused by substances derived from the abnormal nutrition.

In this connection it is well to recall that an abundance of cholesterol is found in many inflammatory and degenerative lesions which are

14. Knack: Arch. f. path. Anat. **220**:36, 1915.

clearly caused by local injuries. The deposition of cholesterol in them takes place long after the primary injury has occurred. The presence of cholesterol in a lesion depends upon the nature of the lesion and not upon the level of the blood cholesterol. For example, the intimal lesions caused by the injection of bacteria into rabbits or those found in the aorta of human beings dying of typhoid fever show as much cholesterol as do the intimal lesions which occur in animals fed an excess of cholesterol, in spite of the fact that the blood in infections contains less than the normal amount of cholesterol.

Knack attempted to answer his query by feeding one group of rabbits an entirely normal diet to which he added very large amounts of cholesterol, and feeding a second group of rabbits an animal diet similar to that used by Ignatowsky. The cholesterol was enclosed in pellets of moistened bread and he was able to feed as much as 4.5 gm. daily in this way. The second group was fed milk, one egg and greens daily resulting in an intake of only 0.3 gm. cholesterol. The milk-egg feeding, in spite of containing much less cholesterol and not being continued so long, produced a well marked atherosclerosis in every rabbit of this group. On the other hand, most of the animals that ate the large amounts of pure cholesterol showed no vascular injury. A few of them exhibited small intimal lesions. He concluded that pure cholesterol when fed with a normal diet does not cause atherosclerosis; and that the milk-egg diet did produce atherosclerosis because such a diet is accompanied by abnormal metabolic products which cause the primary vascular injury.

It would appear, then, in the light of our present knowledge, that cholesterol plays an important part in the formation of the atherosclerotic lesion, but there exists as yet no entirely satisfactory proof that simple hypercholesterinemia is a primary cause of atherosclerosis.

Strain.—Several authors believe that they have produced atherosclerosis by subjecting the arteries to unusual strain. Klotz,[14] suspended rabbits by the hind legs daily for three minutes for as long as 130 days. He reported the occurrence of two types of injury. In those arteries which bore the brunt of the increased pressure, the media showed degenerative changes of the type produced by epinephrin. On the other hand, some of the smaller arteries which felt the increased load less showed atheromatous changes of the intima. Harvey [15] raised the blood pressure of rabbits by digital compression of the aorta below the kidneys. He found changes in the aorta which were entirely analogous to those caused by the injection of epinephrin. Steinbiss [8] tried to duplicate the findings of Klotz and of Harvey by use of their

15. Harvey: Arch. f. path. Anat. **196**:303, 1909.

technic. He, however, added to the strain on the vascular system in both cases. Twelve rabbits were suspended by him for five minutes twice daily for 180 days; and six rabbits for five minutes twice daily for a whole year. These animals accordingly received more than twice the "dose" which Klotz had given them. He also compressed the aorta in twelve rabbits daily for five minutes for 180 days, and in six others for a full year. The result was entirely negative, except for two animals that had been treated by suspension. One of these animals died early in the experiment and the other showed a septic endometritis. Steinbiss reached the obvious conclusion that the lesions found by Klotz and Harvey were caused by something other than the strain on the vessel wall.

Aschoff tried to repeat Klotz' experiments but obtained entirely negative results. Likewise Lubarsh, Starokadomsky, Tsobolow and Fahr failed to obtain vascular lesions by repeating Klotz' technic. In the face of all these failures one is forced to doubt, with Steinbiss, whether experimental atherosclerosis has ever been caused by simple increased strain on the vessel wall.

In summary, it may be said that, of all the methods tried, only the intravenous injection of bacteria and the feeding of animal food has consistently resulted in the production of experimental atherosclerosis. Animal diets are abnormal for rabbits in at least two ways. They contain an excess over herbivorous diets of both protein and cholesterol. A number of workers have tried to prove that the vascular lesions occurring in rabbits fed animal diets were caused by the cholesterol. It has, however, not yet been shown that a simple hypercholesterinemia can produce atherosclerosis. The literature contains no record of a systematic investigation of the possible effect of the protein in the diet on the arteries of rabbits. Our experiments were accordingly undertaken with the intention of establishing the relationships (if any existed) between diets characterized by their high content of protein and atherosclerosis.

RECORD OF FEEDING EXPERIMENTS

Composition of Diets.—Diets containing two different concentrations of protein were fed. Lean beef muscle, mechanically freed of fat and then dried and powdered, was the chief source of protein. Ordinary white bread flour, mixed in equal parts with bran, supplied carbohydrate and roughage. The first diet was made by stirring together 1,000 gm. powdered beef; 2,000 gm. of the flour-bran mixture; 20 gm. sodium chlorid; 50 gm. baking powder and enough water to produce a stiff dough. The latter was then spread thickly in oiled pans and baked in an oven at about 180 C. until a dark brown crust had formed.

The second diet contained 500 gm. powdered beef, 2,000 gm. of the flour-bran mixture, 20 gm. sodium chlorid and 50 gm. baking powder. It was prepared for feeding in the same way as the first diet.

An analysis for nitrogen by the Kjeldahl method of the powdered beef and of the two diets, gave the following values:

100 gm. dried powdered beef.... = 13.0 gm. nitrogen
100 gm. first diet ..... = 5.8 gm. nitrogen
100 gm. second diet ..... = 4.3 gm. nitrogen

Multiplying these nitrogen figures by 6.25 gives the following protein content:

Dried powdered beef..... = 81.2 per cent. protein
First diet ..... = 36.2 per cent. protein
Second diet ..... = 26.8 per cent. protein

In addition to this food, the rabbits were each allowed about 100 gm. of greens once a week.

The animals were obtained from fanciers who had taken special pains to raise them in clean, light, well ventilated pens. During the feeding experiments they lived in pens which had previously been made scrupulously clean and which were kept clean thereafter. Some of the rabbits were housed together in small groups. Many others lived throughout the experiment in individual cages. These cages were located in an unheated shed which protected the animals from wind and rain but which afforded them ample sunshine and ventilation. The date of birth of many of the rabbits was known and we were thus able to determine whether age played a part in the causation of the vascular lesions.

Statistical Outcome.—Twenty-four animals lived for four weeks or more on the diet containing 36 per cent. of protein. They may conveniently be divided into three groups determined by the duration of the experiment. In the first group are ten animals that lived from four to eight weeks on the diet. Two of these rabbits showed very early intimal disease. Six rabbits lived on the diet from ten to sixteen weeks and four of them showed early intimal disease. The remaining eight rabbits ate the high protein mixture from eighteen to thirty-six weeks and all of them presented marked and extensive atherosclerosis.

Fifty-one animals were fed the diet containing 27 per cent. protein. These rabbits fall naturally into two groups—forty that lived less than twenty-six weeks, and eleven that lived from twenty-six to fifty-nine weeks. None of the forty rabbits that lived for less than six months on the diet containing 27 per cent. protein showed atherosclerosis of the aorta; whereas eight of the eleven animals that ate this diet for more than six months presented aortic lesions which were grossly and histologically typical of true atherosclerosis. The vascular lesions in

seven of these eight rabbits were advanced and extensive. The eighth rabbit had eaten the diet for just six months and its aorta showed an early but widely distributed process.

On the basis of these results it is evident, first, that the animals which ate a diet containing 36 per cent. protein showed the atherosclerosis sooner than did the rabbits that received only 27 per cent. protein; and, second, that the occurrence and extent of the atherosclerosis was roughly proportional to the duration of the feeding.

Description of Lesion.—The lesion as seen by the unaided eye is made up of discrete and confluent, raised, opaque white streaks and plaques. The process is always most marked in the upper thoracic aorta and in very early cases may be limited to a few closely set tiny plaques restricted to the arch. As the process becomes older and more marked, the individual lesions are larger and extend further down the aorta. In advanced examples, one of which is shown in Figure 1, the plaques and streaks may be thickly distributed along the whole aorta

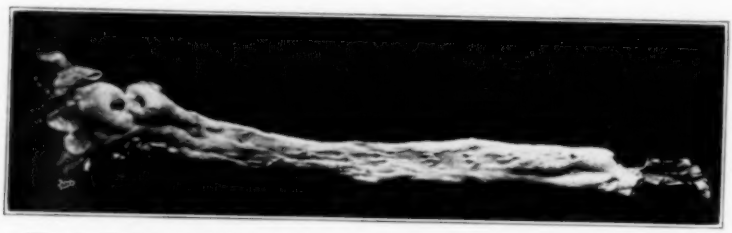


Fig. 1 (Rabbit A-109).—Aorta of a rabbit fed a diet containing 27 per cent. protein for nine months. The photograph shows many large irregular plaques and streaks. The normal intima of the arch is entirely replaced by a broad confluent plaque.

and may also extend a few centimeters into the great vessels given off by the aorta. In the upper aorta the fusion of the plaques often results in the entire disappearance of normal intima. Not infrequently the lesion involves the aortic valves causing them to become thick and stiff. In a few instances the mitral valve was similarly involved.

The lesion when examined with the microscope is seen to be a primary disease of the intima. It begins with a necrosis or fatty degeneration of the endothelial cells (Figs. 3 and 4). The initial injury is very quickly followed by great thickening of the intima due to the presence in it of large cells loaded with fat and imbedded in a hyperplastic elastic tissue (Fig. 5). At a somewhat later stage, when repair is being attempted, the picture has added to it a broad band of fibrous elastic tissue on the lumen side of the vessel (Fig. 6). Below this is the area of necrosis and fatty degeneration which by now has invaded

Fig. 2.—Normal aorta; hematoxylin and eosin: *A*, narrow intima; *B*, broad muscular media; *C*, adventitia.

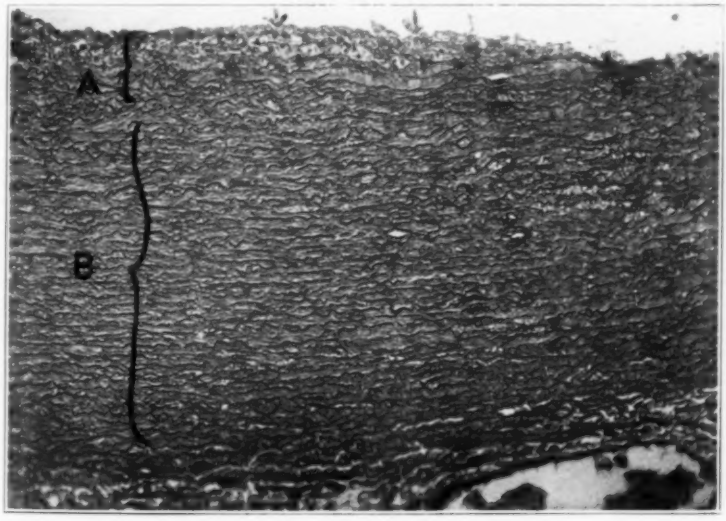


Fig. 3 (Rabbit A-141).—Aorta of a rabbit fed a diet containing 36 per cent. protein for eight weeks. Hematoxylin and eosin: *A*, intima showing the early stages of atherosclerosis; *B*, media.

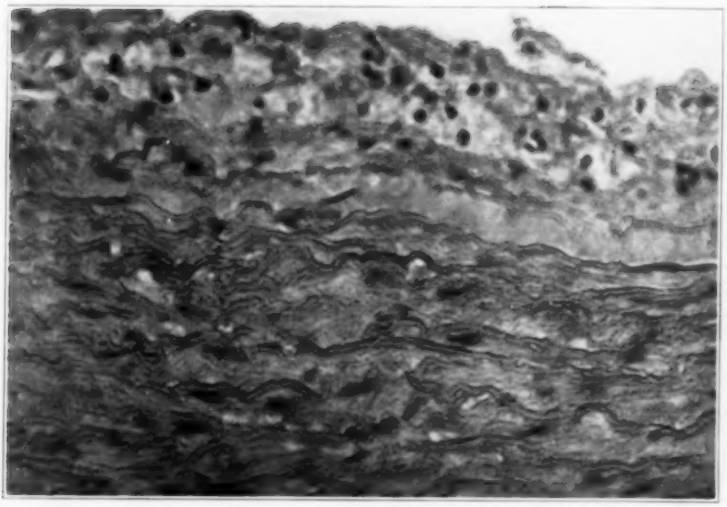


Fig. 4.—A high power view of the portion of the intima in Figure 3 indicated by the arrows. The intima cells show swelling, hyaline degeneration and vacuolization. Their nuclei are pyknotic. Fat stains demonstrated that many of the vacuoles were filled with fat.

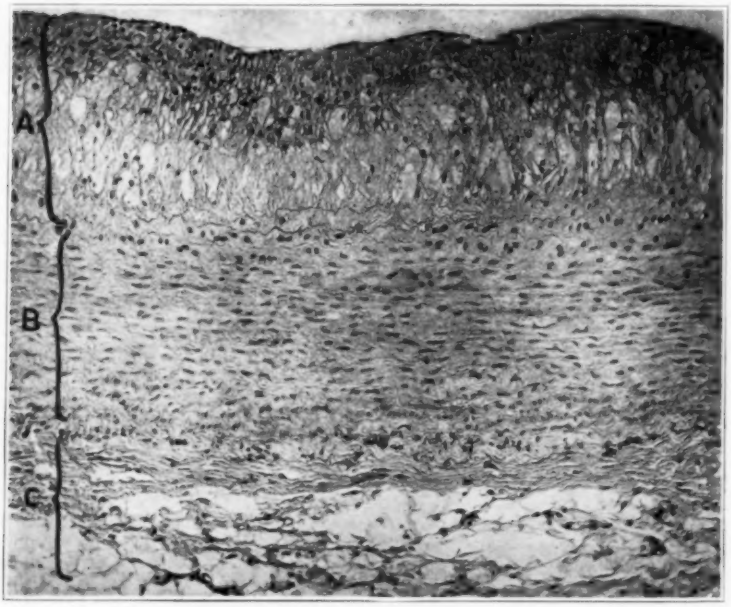


Fig. 5 (Rabbit A-27).—Aorta; hematoxylin and eosin: Atherosclerosis moderately advanced. The rabbit was fed a diet containing 27 per cent. protein for nine months. *A*, the great thickening of the intima brought about by the large vacuolated cells and the hyperplastic elastic tissue; *B*, normal media; *C*, adventitia.

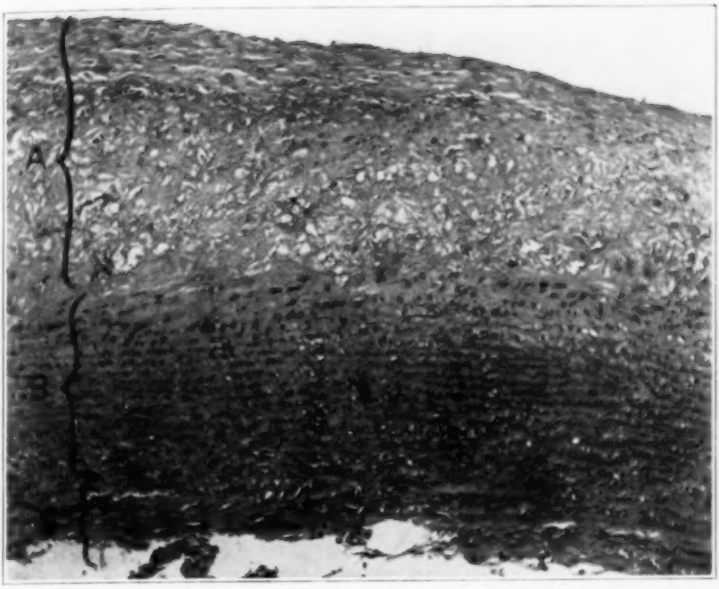


Fig. 6 (Rabbit A-108).—Aorta; hematoxylin and eosin: Atherosclerosis is far advanced. The rabbit was fed a diet containing 27 per cent. protein for eleven months. *A*, intima showing a band of dense fibrous tissue on the lumen side of the vessel and degenerated fat containing cells below; *B*, the degeneration has progressed far into the media; *C*, adventitia.

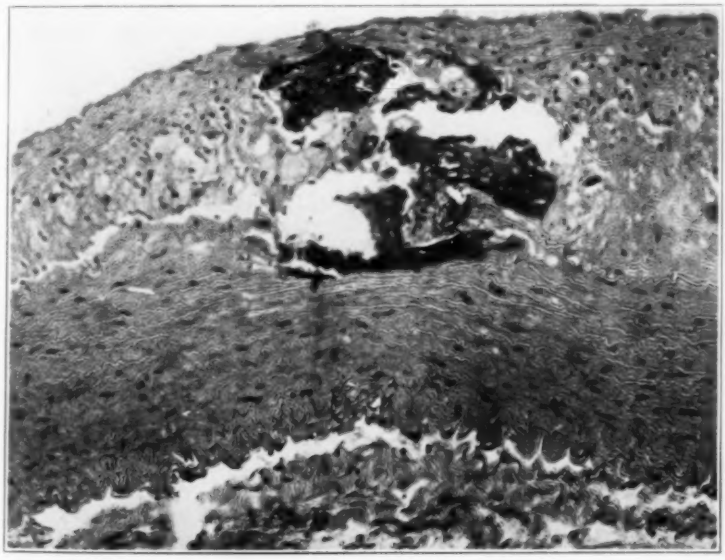


Fig. 7 (Rabbit A-109).—Abdominal aorta of a rabbit fed a diet containing 27 per cent. protein for nine months. Hematoxylin and eosin: The microphotograph shows a large irregular mass of calcium deposit involving most of the intima in this area and dipping down into the media.

the media to a variable extent. At this stage in the process, amorphous masses of calcium may be found scattered irregularly through the degenerated portion of the lesion (Fig. 7). When portions of the aorta fixed in formaldehyd are examined by means of frozen sections and stains for fat, a convincing demonstration of the enormous amount of lipoid material present in the intima is obtained (Fig. 8). The hyperplasia and fraying of the intimal elastic tissue so characteristic of this lesion is well shown in Figure 9 which is a microphotograph colored by an artist from the original.

The experimental production of atherosclerosis of the coronary arteries is of special interest because it offers another method for use

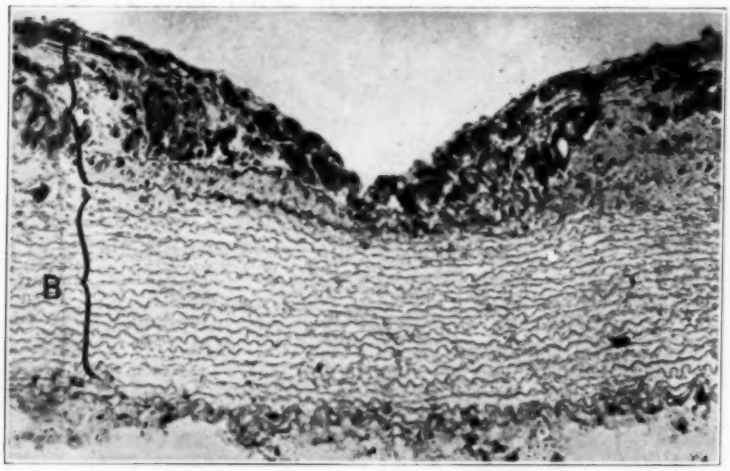


Fig. 8 (Rabbit A-27).—Aorta; frozen section; sharlach R. *A*, the black points and the irregular dark masses represent the lipoid substances present in the intima; *B*, media.

in the study of the etiology of myocarditis. The demonstration of an atherosclerotic process involving a small branch of the left coronary artery is presented in Figure 10.

CONTROLS

It has already been pointed out that the occurrence and extent of the atherosclerosis were roughly determined by the amount of protein in the diet and by the duration of the feeding. Such facts strongly suggest that the vascular lesions were produced by the high protein diets; but before being certain that such is the case it is necessary to investigate several possible sources of error.

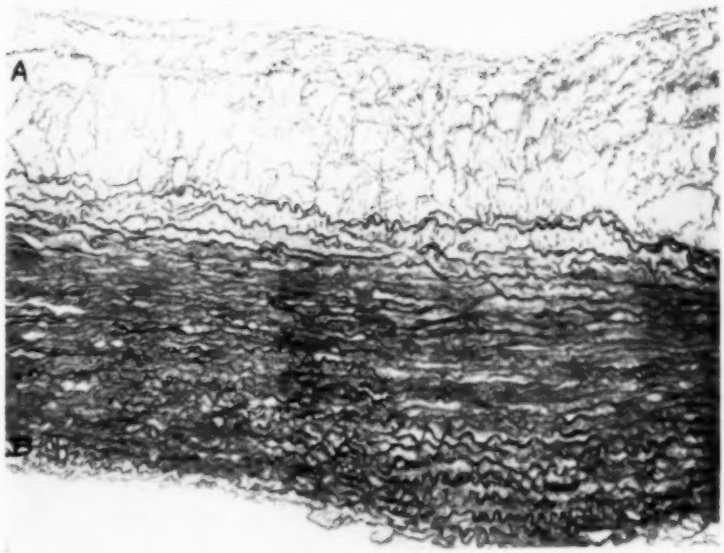


Fig. 9 (Rabbit A-88).—Aorta; Weigert's elastic tissue stain: *A*, the thickened intima showing the hyperplasia and fraying of the elastic tissue; *B*, media.

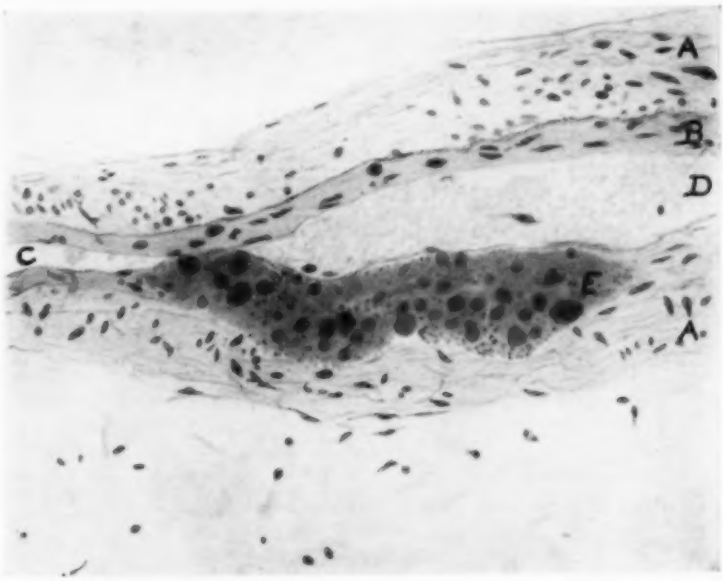


Fig. 10.—Myocardium showing a small artery; fat stain: *A*, the media; *B*, the somewhat thickened intima. The area marked E.E. shows extensive fatty degeneration of the intima and involvement of the underlying media by the same process; *C*, indicates the lumen of the vessel, and *D*, a mass of blood cells lying in the lumen.

Spontaneous Atherosclerosis.—Until it is known how often primary intimal disease will be found in control rabbits, it is, at the very least, hazardous to attribute the atherosclerosis of the experimental animals to the diets. A number of investigators have obtained information in regard to this question.

Steinbiss [8] has examined the aortas of more than 500 rabbits. The group contained not only the laboratory animals but in addition many rabbits shot by hunters. With the exception of two questionable cases, he found no spontaneous atherosclerosis.

Loeb [16] examined 483 normal rabbits carefully without finding any arterial changes.

E. C. Rosenow [17] has noted the condition of the first portion of the aorta in 1,548 rabbits. There were atheromatous patches in only three. An additional 300 rabbits has recently been subjected to necropsy by him. Atherosclerosis of the beginning of the aorta was not found in a single instance.

Miles,[18] on the other hand, found aortic lesions in seventeen of forty-nine (34.6 per cent.) supposedly normal rabbits. She adds that rabbits obtained from a dealer raising them for the market in large numbers and in close quarters showed a higher percentage of lesions than those obtained from other sources. The disease as described by her involved the media primarily and chiefly; and was identical with the epinephrin effects. Such necroses of the media have been frequently noted by several other investigators. They should not be confused with atherosclerosis and give us no information in regard to the spontaneous occurrence of disease of the intima in rabbits.

Levin and Larkin (quoted by Steinbiss [8]) report finding spontaneous atherosclerosis in 13 per cent. of rabbits. The material consisted of animals that had been injected with blood serum and "nontoxic substances." Since the nature of the "nontoxic substances" is not mentioned, one may still question whether some of these substances were not after all toxic for the blood vessels. It may also be questioned whether the injection of blood serum may not result in the formation of substances injurious to blood vessels. In any case, there is no more ground for using such material as a control than there would be if it were presented as evidence that the substances injected by Levin and Larkin were injurious to the blood vessels.

We have carefully examined the aorta in 116 rabbits obtained from several sources. Twenty of these animals had been used in the laboratory course in pharmacology to demonstrate the action of drugs. The aortas of all of these rabbits were normal. Seventy-seven rabbits were

16. Loeb: Deutsch. Med. Wchnschr. **39**:1819, 1913.
17. Rosenow: Personal communication.
18. Miles: J. A. M. A. **49**:1173 (Oct. 5) 1907.

obtained from the hospital laboratory. Many of them had been bled to death, the blood being used to make culture mediums. A few of them had received injections of bacteria some weeks before they were killed. A few others were found dead in the stock cages. Of these seventy-seven rabbits, four that had been used for bleeding, showed abnormalities of the upper aorta. The remaining seventy-three rabbits presented normal aortas.

Effect of Laboratory Environment.—Nineteen of the 116 control rabbits formed a special group. These animals were set aside in the ordinary laboratory pen for the purpose of answering two questions: (1) whether the conditions prevailing in our laboratory were, when long continued, in themselves a cause of atherosclerosis, and (2) whether age was a factor in the production of atherosclerosis. These rabbits were given the same food as the stock animals. No special precautions were taken to protect them from infection. They were, on the contrary, exposed to the infections brought in by newcomers in adjoining cages. When one of these control rabbits became infected, it was still left in the pen with the other controls. Three of these nineteen rabbits had been injected with bacteria before being placed in the control pen. One had received six intravenous injections of diphtheria bacilli; the second one received repeated injections of dead typhoid bacilli and the third one (A 83) received six intravenous injections of *Streptococcus hemolyticus*. This animal lived in the control pen for a year following the injections. He underwent a progressive emaciation with a fall in weight from 2,250 gm. to 1,380 gm. during the year. For many weeks preceding his death he suffered from very severe "mange" accompanied by ulceration of the skin. His aorta was normal, except for the presence in the arch of several pale yellow wartlike papules just visible to the naked eye.

The rabbit that received the injections of diphtheria bacilli lived in the control pen nine months. He also showed a progressive emaciation and severe mange. His weight fell to 1,110 gm. His aorta was normal.

The rabbit injected with dead typhoid bacilli died after being in the control pen for five months. At the necropsy a caseous pneumonia of the upper and middle lobes of the right lung and an extensive fibrous pericarditis was found. His aorta was normal.

Sixteen of these nineteen rabbits were placed in the control pen when they were about 6 months old. Three of them died within six weeks; three lived from two to four months; three lived six months; three lived more than six months but less than one year; four lived more than one year; fifteen died directly or indirectly of infection. Acute pneumonia killed a few of them. Protracted "snuffles," accompanied by marked wasting and often complicated by bronchopneumonia, caused the death of many of the rabbits. None of them showed lesions of the aorta.

We wish to emphasize the point that these fifteen rabbits died of the infections to which all the animals in the laboratory were exposed. Some of them were killed quickly but a majority of them bore their infection a number of months. Nevertheless, no disease of the aorta was seen in any of them. We have accordingly reached the conclusion that the infections which were prevalent in our laboratory during the period of our experiments were not a cause of atherosclerosis, even though some of these infections were chronic in type and remained active for months. This statement may occasion some surprise since there are good reasons for believing that pathogenic bacteria cause atherosclerosis in rabbits; but in spite of this it is a fact that the types of spontaneous infection from which these fifteen rabbits suffered, did not cause vascular disease in them.

The special group of nineteen rabbits used to discover whether conditions in the laboratory were such as to be a cause of atherosclerosis contained only one animal whose aorta was not normal. This was rabbit A 83, already mentioned. The microscopic examination of the small wartlike papules seen on the surface of the aorta, showed that they consisted of fibrous connective tissue. No hyperplasia of the elastic lamina was visible and no fat was present in the lesion. These are the features of a healed process. Since this animal had received intravenous injections of the hemolytic streptococcus a year before his death, the streptococcus may have caused the aortic lesion. At all events, this is an adequate explanation for the lesion, which accordingly need not be attributed to any conditions arising after this rabbit was placed in the special control group.

Since eighteen of these nineteen rabbits showed no vascular disease, even though most of them had lived in the laboratory for many months in such a way as to receive no special care or protection, it is evident that the laboratory conditions were not such as to cause atherosclerosis.

Calcification of Media.—There were four rabbits, in addition to A 83, among the control group of 116 (3.4 per cent.) whose aortas were abnormal. The vascular lesions found in these animals were, without exception, very small in extent. They consisted of one or several tiny papules rising above the surface of the intima in the ascending limb or arch of the aorta. They were so small that they were often seen by the unaided eye only when especially sought for.

Among the treated animals, four of those that were fed the diet containing 27 per cent. protein, and two of those that received the food containing 36 per cent. protein—a total of six out of eighty-two (7 per cent.)—showed vascular lesions of this same type.

These abnormalities of the aorta were easily distinguished from the primary intimal disease which occurred in the animals fed the high protein diets. In the former, the lesions were always restricted to the

uppermost part of the aorta and bore no special relation to the openings of the small vessels. The individual lesions were always small, at most 2 or 3 mm. in diameter, whereas in the latter the lesions were much larger, took the form of large irregular plaques and broad bands, were often widely distributed along the thoracic aorta and showed a striking tendency to be most marked around the openings of the small vessels.

On microscopic examination, it was found that these lesions were, without exception, situated in the media. Bands of muscle cells were necrotic and very often calcified. The process appeared to have a

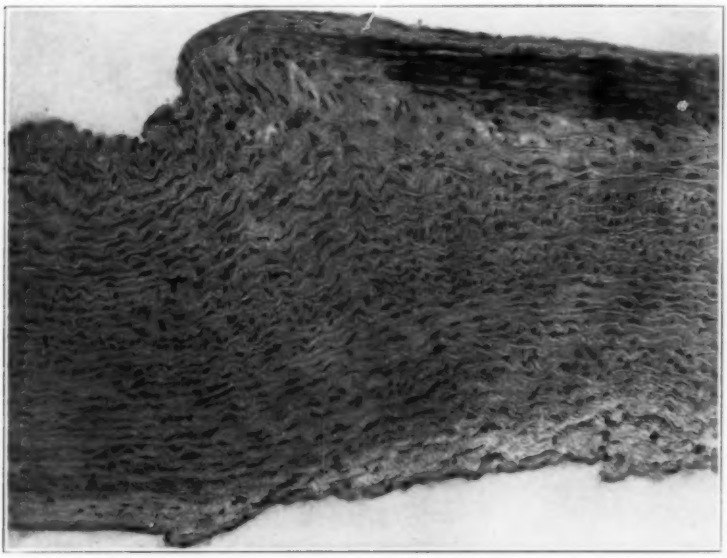


Fig. 11 (Rabbit A-2).—Control; aorta: The upper media shows calcified muscle cells. The overlying intima is normal.

predilection for the upper media but did not extend into the intima. A typical example of this condition is represented by Figure 11. The absence of fat in this medial lesion, and its abundance in the atherosclerosis of the meat-fed animals, was alone sufficient to make a sharp differentiation between these two processes.

On the basis of the experience of other investigators, and as a result of the careful study of our own 116 control rabbits, we have concluded that: (1) in any large series an occasional supposedly normal rabbit may be expected to show small restricted vascular lesions; (2) these lesions can, by means of the microscope and special strains, easily be

distinguished from the extensive fatty intimal disease seen in rabbits that have eaten the experimental diets for some weeks; (3) long exposure to the conditions existing in our laboratory does not cause atherosclerosis.

Age.—In the case of man, age is an important predisposing factor in the causation of atherosclerosis. It might be argued that this also held true for the rabbit and that our rabbits were atherosclerotic because they were old. An answer to this question may be obtained by comparing the ages at death of rabbits of known age in the control group that had lived in the laboratory for more than six months with similar rabbits that had eaten a high protein diet for six months or more. Table 1 shows that the control rabbits whose aortas were normal were as a group older than the atherosclerotic rabbits, and that the three rabbits which had not developed atherosclerosis, even though they had eaten the high protein mixture for more than six months, were, on the

TABLE 1.—LACK OF RELATION BETWEEN AGE AND ATHEROSCLEROSIS

Ate Diet Containing 27 per Cent. Protein for Six Months or More				Controls That Lived in the Laboratory More Than Six Months	
Number	Normal Aorta, Age at Death	Number	Atherosclerosis, Age at Death	Number	Normal Aorta, Age at Death
A-61	62 weeks	A-67	50 weeks	A- 1	41 weeks
A-78	68 weeks	A-27	56 weeks	A- 5	42 weeks
A-60	87 weeks	A-26	61 weeks	A-74	46 weeks
		A-61	62 weeks	A-81	58 weeks
		A-77	70 weeks	A-12	61 weeks
				A-37	66 weeks
				A-52	73 weeks
				A-71	84 weeks
				A-84	92 weeks
Averages.....	72 weeks	.....	60 weeks	.....	63 weeks

average, older than the animals which showed the aortic disease. It is evident that the atherosclerosis found in the meat eating rabbits was not caused by old age.

Infection.—It has already been shown that the infections which were prevalent in the laboratory did not produce atherosclerosis in rabbits to which normal food was fed. It might, however, be suspected that the high protein diet could produce metabolic disturbances which would make it possible for such laboratory infections to injure the intima; or that such a diet could break down the resistance (possessed by normal rabbits) to those bacteria in themselves capable of causing atherosclerosis. One would expect to be able to detect the presence of such hypothetic metabolic disturbances by their impress on the general condition of the animals. An opinion regarding an animal's general condition may be obtained by observing its coat and its conjunctivae; the rate at which it grows; its maximum weight. Our high protein rabbits presented the smooth coat and bright eyes of health and in this

respect appeared more nearly normal than the controls. Table 2 presents the maximum weights attained by rabbits fed a diet containing 27 per cent. protein and by controls, all of which had lived in the laboratory at least six months. It will be seen that the high protein rabbits reached a much greater adult weight than the controls. Three of the rabbits eating the high protein mixture were killed at a time when they presented the appearance of perfect health. Two of them gave the most

TABLE 2.—MAXIMUM WEIGHT AND MODE OF DEATH OF RABBITS LIVING IN THE LABORATORY SIX MONTHS OR MORE

Fed Diet Containing 27 per Cent. Protein						Normal Diet		
Became Atherosclerotic			Aorta Remained Normal			Aorta Was Normal		
Number	Maximum Weight, Gm.	Mode of Death	Number	Maximum Weight, Gm.	Mode of Death	Number	Maximum Weight, Gm.	Mode of Death
A-77	2,770	Found dead	A-78	2,750	Lethargy; killed	A-1	1,670	Found dead
A-27	2,600	Found dead	A-118	2,670	Severe "mange"; dying, killed	A-5	1,850	Diarrhea; found dead
A-67	2,040	Lethargy; killed	A-61	2,600	Found dead	A-12	1,550	Found dead; bilateral purulent pneumonia
A-109	2,850	Dying; killed	A-60	2,800	Appearance of health; killed	A-74	1,450	Found dead
A-88	2,040	Lethargy; killed				A-68	1,290	Severe snuffles; found dead
A-26	2,640	Found dead				A-84	1,380	Dying; killed
A-100	3,270	Appearance of robust health; killed				A-52	2,750	Dying; killed; bronchopneumonia
A-108	3,700	Appearance of robust health; killed				A-71	3,000	Mange; found dead
						A-37	1,450	Found dead
						A-81	1,600	Found dead
Averages..	2,770	.....	.....	2,700	.....	.....	1,812	

extensive examples of atherosclerosis in the series. They were also the heaviest rabbits in the series.

We were thus unable to obtain any support for the idea that nutritional disturbances attendant on the high protein diet had resulted in the invasion or growth of bacteria. But our evidence is of a negative sort. It is impossible to prove absolutely that bacteria had no part in the causation of this lesion. It is, however, a fact that the bacteria which infected the control rabbits and eventually killed all but one of them were incapable of producing atherosclerosis in rabbits which received normal food. Hence, it must be true that the high protein

diet was the determining factor in the occurrence of the atherosclerosis. If bacteria or their toxins had anything to do with the etiology of this disease, their contribution must have been of a secondary and relatively unimportant nature.

Cholesterol.—The possible place of cholesterol in the etiology of experimental atherosclerosis has already been discussed. It will be recalled that several investigators held the view that the atherosclerosis which occurred in rabbits fed various kinds of abnormal diets was in every instance caused by the cholesterol of the diet. Wesselkin,[19] who fed one egg yolk daily (cholesterol content, 64 mg.) for six months; Starokadomski[20] who fed two egg yolks daily for nine weeks, and Saltykow[21] who fed 400 c.c. milk (cholesterol content, 48 mg.) for more than one year, were each convinced that the cholesterol in the food was responsible for the intimal disease. These were the smallest doses used by students of this question who obtained positive results. It is of interest to compare the cholesterol content of our diet[22] with these minima. One hundred grams of dried powdered beef yielded 190 mg. cholesterol. Since the average twenty-four hour urinary nitrogen of rabbits on the diet containing 27 per cent. protein was 2 gm., they must have obtained about 12 gm. protein daily from the diet. Most of this protein was derived from the beef and it has higher cholesterol values than the other ingredients of the diet. If all of the protein had come from the beef (protein 82 per cent.), each rabbit would have absorbed about 15 gm. of dried beef daily and this would have made possible a maximum of 28 mg. cholesterol daily. Hence the amount of cholesterol received by our rabbits was 22 per cent. of the amount fed by Starokadomski, 44 per cent. of that used by Wesselkin, and 58 per cent. of that eaten by Saltykow's rabbits. The amount of cholesterol ingested by our rabbits was accordingly much less than the smallest amounts said to have caused atherosclerosis.

Saltykow fed about twice the amount of cholesterol contained in our diet. His series consisted of six rabbits that received daily 400 c.c. milk each. The animals lived from one to two years and two and one-half months. Three rabbits showed extensive atherosclerosis and one presented no intimal disease. On the other hand, eight of our eleven rabbits which ate a high protein diet with its daily dose of 28 mg. cholesterol developed widespread atherosclerosis in from six months to one year. Even if it were admitted that the 48 mg. cholesterol contained in Saltykow's diet had caused advanced atherosclerosis in 50 per cent. of the animals that ate this diet one year or more, this would not prove, or even strongly suggest, that the extensive intimal disease found

19. Wesselkin: Rusky Vrach, p. 1651, 1912.
20. Starokadomski: Frankfurt Ztschr. f. Path. **3**:912, 1909.
21. Saltykow: Arch. f. path. Anat. **213**:8, 1913.
22. We are grateful to Dr. H. G. Waller for the cholesterol determinations.

in 73 per cent. of our rabbits that had received about half this amount of cholesterol daily for about half the length of time was caused by the cholesterol.

In answer to the criticism that the vascular disease found in the milk-fed rabbits was not caused by cholesterol, Saltykow pointed out that Wesselkin had fed a total of 11.52 gm. cholesterol per rabbit in six months; Starokadomski a total of 8.064 gm. in nine weeks; whereas his own total was from three to five times greater—about 37 gm.—because of the duration of his experiments.

If we apply this same type of calculation to our experiments, it is found that the average total dose of cholesterol was 7.56 gm. for the eleven rabbits that ate the diet containing 27 per cent. protein from six months to one year; and 6.993 gm. for a group of eight rabbits all of which developed marked atherosclerosis within six months on a diet containing 36 per cent. protein. Hence, the total dose received by our rabbits was less than that used by Wesselkin or Starokadomski and much less than that fed by Saltykow.

Since both the daily and the total dose of cholesterol received by our rabbits was so much less than that considered by other investigators necessary to cause atherosclerosis, it does not seem likely that the cholesterol in our diets played an important rôle in the etiology of the atherosclerosis which we are reporting.

SUMMARY

The term atherosclerosis should be applied only to primary disease of the intima characterized by fatty degeneration and hyperplasia of the elastic tissue. Investigators have obtained this lesion experimentally by injecting bacteria and by feeding abnormal diets. Many students of experimental alimentary atherosclerosis believe that the cholesterol contained in the abnormal diets is the sole cause of the intimal disease. The reasons why we do not concur in this view have been discussed. Many of the experimental diets used by earlier workers were abnormally high in protein, but this factor received no special study. Our experiments have shown that the prolonged ingestion of excessive amounts of protein by rabbits will result in extensive atherosclerosis of the aorta and several other arteries. Study of sources of error has demonstrated that the atherosclerosis found in the rabbits that ate the high protein diets was not spontaneous; was not the effect of laboratory environment, age or infection; could easily be distinguished from the spontaneous calcification of the media; and was not caused by the small amount of cholesterol in the diet.

THE PRESENCE OF AUER BODIES IN LEUKEMIC TISSUES *

MAURICE N. RICHTER, M.D.

NEW YORK

Since the discovery by Auer,[1] in 1906, of certain rodlike bodies in the red blood cells in a case of acute leukemia, several additional articles have appeared, dealing with the character of these bodies and the type of cell in which they are found. Inasmuch as the literature on the subject is still meager, the report of an additional case may be of some value. The case is of special interest since it is the first, as far as can be ascertained, in which the Auer bodies have been demonstrated in microscopic sections of the various organs.

A résumé of the literature reveals little that was not described in Auer's report. Auer noted that rod-shaped bodies were visible as refractive objects in the cytoplasm of from 6 to 10 per cent. of the "large lymphocytes," in preparations of fresh blood; that they were stained clearly (red) by Wright's and Leishman's stains, faintly by Ehrlich's triacid mixture, but not by Ehrlich's haematoxylin and eosin, methylene blue and eosin, Loeffler's methylene blue, sudan III or Lugol's solution. The rods were found free in the blood only in the vicinity of disintegrated cells, and once were seen in the cytoplasm of a normoblast.

Similar findings were later reported by other authors. Pappenheim,[2] Pappenheim and Hirschfeld,[3] Ottenberg,[4] Isaac and Cobliner,[5] and Naegeli[6] found Auer bodies in "large lymphocytes." Ottenberg inoculated monkeys with fresh blood and with splenic pulp, but neither transmitted the Auer bodies nor produced leukemia. Roth[7] reported Auer bodies in a case of subacute miliary tuberculosis with certain symptoms that may be interpreted as leukemic. The blood in his case,

---

* From the Pathological Laboratories of Bellevue and Allied Hospitals.

1. Auer, J.: Some Hitherto Undescribed Structures Found in the Large Lymphocytes of a Case of Acute Leukemia, Am. J. M. Sc. **131**:1002, 1906.

2. Pappenheim, A.: Ueber eigenartige Zelleinschlüsse bei Leukämie, Berl. klin. Wchnschr. **45**:60, 1908.

3. Pappenheim and Hirschfeld: Ueber akute myeloide und lymphadenoide makrolymphozytäre Leukamie an der Hand von zwei verschiedenen Fallen, Folia haematol. **5**:347, 1908.

4. Ottenberg, R.: Observations on Acute Leukemia, with Special Reference to Auer's Bodies, Am. J. M. Sc. **138**:562, 1909.

5. Isaac and Cobliner: Ueber mikrolymphozytäre Typen akuter myeloischer Leukämien, Folia haematol. **10**:459, 1910.

6. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, Ed. 2, Leipzig, Veit u. Comp., 1912, p. 482.

7. Roth, O.: Ueber einen bemerkenswerten Blutbefund bei einem Fall von subakuter Miliartuberkulose, Ztschr. f. klin. Med. **78**:75, 1913.

as in the one reported herewith, contained a high percentage of "myeloblasts" in which the Auer bodies were found. Chosrojeff [8] also found Auer bodies in "myeloblasts," and thinks it possible that in earlier cases cells reported as large lymphocytes may have been myeloblasts. In this case Auer bodies were found in smears of the bone marrow, spleen and lymph nodes, but no mention is made of their presence in tissue sections. Rosenthal [9] found Auer bodies in "myelogones" and noted that they gave a positive oxidase reaction. Thro [10] reported red staining rod-shaped granules in "myeloblasts" but gives no further description of their morphology and staining reactions. It appears from his illustrations that they may have been Auer bodies. Nakajima,[11] Yoshida,[12] and Ishikawa [13] also reported finding Auer bodies in myelogenous leukemia. Ishikawa tried numerous stains on blood smears, and found the bodies well stained by the Romanowsky methods, poorly stained by Ehrlich's triacid or eosin stain, positively stained in the indophenolblue synthesis, and stained blue by pyronin. They were vitally stainable without metachromatism by gentian violet and safranin. They did not stain with sudan III, Loeffler's methylene blue, Gram stain, Gabbett's stain for tubercle bacilli, or the Levaditi silver method. In tissues fixed in liquor formaldehyd, cut by the paraffin, celloidin and frozen section methods and stained by hematoxylin and eosin, Giemsa and oxidase stains, no Auer bodies were seen. In Ishikawa's cases, aerobic and anaerobic cultures of blood were negative, and inoculation of mice, rabbits, guinea-pigs and apes gave negative results. Here, too, the leukemia was of the myeloblastic type.

REPORT OF CASE

History.—The patient, an Italian woman, aged 23, was admitted to the wards of the Fourth Medical Division, Bellevue Hospital, with the following history:

Two weeks before admission, she experienced pain behind the sternum, sore throat, nonproductive cough, dyspnea, headache and fever. During the last four days these symptoms became worse, the gums became swollen and tender, and a swelling appeared on the right side of the face and neck.

Physical Examination.—On examination, the woman appeared to be acutely ill. The mucous membranes were pale, and the skin was lemon-yellow. There was a tender swelling over the right side of the jaw and neck, without redness or fluctuation. The cervical lymph nodes were enlarged and tender. The throat was congested, the gums were swollen and tender, especially on the right side, and there was a cavity in one molar tooth. There were hemorrhages in both

8. Chosrojeff, G. P.: Myelosis Aleukaemica Acuta Micromyeloblastica, Folia haematol. **20**:33, 1915.

9. Rosenthal, N.: Studies on the Oxidase Reaction of the Cells in Normal and Leukemic Blood, Arch. Int. Med. **20**:184 (Aug.) 1917.

10. Thro, W. C.: Some Unusual Blood Cells in Diseases of Bone Marrow Origin, J. M. Research **38**:385, 1918.

11. Nakajima: Quoted by Ishikawa.[13]

12. Yoshida: Quoted by Ishikawa.[13]

13. Ishikawa, T.: Ueber den Auerkörper bei einen Falle von akuter Myeloblastenleukämie, Mitt. a. d. Med. Fakult. d. k. Univ. zu Tokyo **22**:321, 1919.

eye grounds. The spleen was not palpable and the lymph nodes, with the exception of the cervical nodes, were not enlarged. The rest of the history and physical examination was unimportant.

Laboratory Findings.—The urine was negative. No Bence-Jones body was found on two examinations. The single blood culture made was sterile. Blood count: On admission, the red cells numbered 1,900,000; the hemoglobin, 30 per cent. The red cells rose to 4,000,000 and the hemoglobin to 65 per cent. after transfusion, but rapidly declined again. The white cells on several examinations were nearly constant, numbering between 6,000 and 8,000. Differential counts are given in the accompanying table.

TABLE OF DIFFERENTIAL COUNTS *

Date	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Neutrophil Myelocytes	Eosinophil Myelocytes	Myeloblasts†	Per Cent. Myeloblasts with Auer Bodies‡	Plasma Cells
2/16/22	2.6	28.6	0	0.4	5.2	0.2	63.0	32.0	0
2/17/22	5.2	31.3	1.1	0	1.0	0.2	60.0	30.7	1.2
2/24/22	3.9	8.6	0	0	7.2	0.8	79.5	36.8	0
3/ 3/22	2.4	18.3	3.7	0.4	7.8	0	66.6	28.1	0.8

* The counts were made on smears stained with Wright's or Jenner-Giemsa stains, and are based on counts of at least 500 cells.

† These cells were atypical. The term "myeloblast" is used to indicate a cell in an early stage of myeloid differentiation, before definite granules can be demonstrated with ordinary stains (vide infra).

‡ The highest percentage of myeloblasts containing Auer bodies previously reported was by Ishikawa,[13] from 17 to 19 per cent. They are often found in much less than 1 per cent. of the myeloblasts.

Subsequent Course.—About two weeks after admission, the spleen became palpable and remained just below the costal margin. Five weeks after admission, a peritonsillar abscess developed and was opened. The temperature oscillated between 99 and 102 F., except during a chill following transfusion, when it rose temporarily to 105.8 F., and during the last few days of life, when it remained nearly constant at 104 F. The patient died at the end of her fifth week in the hospital.

NECROPSY FINDINGS

Macroscopic Examination.—The changes found at necropsy were not marked. Except for an enlarged spleen and a few petechial hemorrhages in the larynx and ileum, the gross findings were not remarkable. The spleen weighed 325 gm. and presented, on section, numerous lymph follicles of normal size.

Microscopic Examination.—Microscopic examination of the organs showed the presence of myeloblasts in the blood vessels, but no leukemic nodules or infiltrations. Tissues were fixed in Helly's fluid and in liquor formaldehyd, sectioned by the celloidin, paraffin and frozen section methods, and stained with hematoxylin and eosin, Dominici's stain, azure II and eosin, Giemsa, Wright's blood stain, Goodpasture's acid polychrome-methylene blue and eosin,[14] Gabbett's stain, Gram's stain, and Graham's[15] oxidase method.

In all of these stains except the hematoxylin and eosin stain, Gabbett's stain and Gram's stain, Auer bodies were distinctly seen in some of the myeloblasts. The various methods were not equally satisfactory for their demonstration,

14. Goodpasture, E. W.: An Acid Polychrome-Methylene Blue Solution for Routine and Special Staining, J. A. M. A. **69**:998 (Sept. 22) 1917.

15. Graham, G. S.: The Oxidising Ferment of the Myelocyte Series of Cells and Its Demonstration by an Alphanaphthol-Pyronin Method, J. M. Research **35**:231, 1916.

however, as the bodies were more numerous, larger, and very much more distinct in the oxidase preparations (in which they reacted positively) than in any other (Fig. 3). Goodpasture's, Giemsa and Wright's blood stains, following Helly's fixation, were also very satisfactory. In azure-eosin or Dominici preparations fewer Auer bodies were seen.

(*a*) The Bone Marrow: Sections from the vertebrae and ribs contained the normal marrow elements, but the myeloblasts were somewhat increased in number, some of them containing Auer bodies.

(*b*) The Spleen: The spleen was the most interesting of the organs. Its fundamental structure was preserved. The follicles were normal in size, and presented two distinct zones: a central portion composed of typical small and medium sized lymphocytes, and a peripheral portion the cells of which were larger, with paler nuclei and a pale lavender cytoplasm.

The splenic pulp was very cellular. In the blood sinusoids were numerous myeloblasts, some of which had apparently attached themselves to the vessel walls and wandered out by ameboid movement. Numerous examples of myeloblasts half within and half outside of the blood vessels were visible, some of them containing Auer bodies.

In addition to the myeloblasts were small groups of typical plasma cells, with transitional stages between them and lymphocytes. There were also occasional eosinophilic myelocytes.

The endothelium of the sinusoids was intact, the cells of the smaller vessels bulging sharply into the lumen, as is normal.

The myeloblasts of the pulp were closely grouped around the follicles. There was no admixture of follicular and pulp elements, however, the zone of contact being sharply defined. This perifollicular accumulation of myeloblasts was best seen with the oxidase stain, the follicles themselves reacting negatively and the perifollicular zone positively.

The splenic pulp (and lymph node) also contained a number of globular bodies, varying in diameter from about 1 to 15 microns. The smaller ones occurred in plasma cells. The larger globules so distorted the cell that its nature could not be determined. As a rule, there were groups of globules, with considerable variation in size within the group. With Dominici's stain, the globules were purple; with Gram's method they retained the dye; in the oxidase preparations they reacted positively and appeared as homogeneous structures.

(*c*) Lymph Node: Sections of a lymph node showed that some of the follicles were relatively normal. In others there were large numbers of plasma cells, a few of them containing purplish globules. When the cells were filled with these globules, they closely resembled the "mulberry cells" of Weber.[16] They appeared to be the same as those seen in the splenic pulp.

The other organs presented no important changes, except for the presence of myeloblasts in the blood vessels, some with Auer bodies.

DISCUSSION

From the standpoint of diagnosis, an important feature of the blood picture is the high percentage of myeloblasts and all transitional stages to the myelocyte. This, with the normal total white count, places the case clinically in the class of "aleukemic myeloblastic leukemia" or "acute aleukemic myelosis."

---

16. Weber and Blendinger: A Note on "Mulberry Cells" and Clusters of Eosinophil Spherules, Probably a Form of Russell's "Fuchsine Bodies" in the Wall of a Chronic Cerebral Abscess and in a Case of Multiple Myeloma, J. Path. & Bacteriol. **11**:59, 1906.

Gorham [17] has critically reviewed the reported cases of acute myelogenous leukemia, and gives five essentials for diagnosis: (1) An aleukemic or subleukemic stage. (2) An acute downward course with death usually ensuing in one to four months. (3) Characteristic blood picture of myeloblasts and myelocytes, with transition forms between the two. (4) Typical gross and histologic findings in the liver, spleen, bone marrow and lymph nodes. (5) Specific proof of myeloid elements by enzyme reactions.

The present case easily satisfies four of these points. There is room for argument, however, in that grossly and microscopically the pathologic changes were not very marked. It must be remembered that histologic changes similar to those of leukemia, but less extensive, may occur in acute infections.

The Myeloblasts.—Since Auer bodies have been described in "large lymphocytes," "myelogones," and "myeloblasts," it is important in the

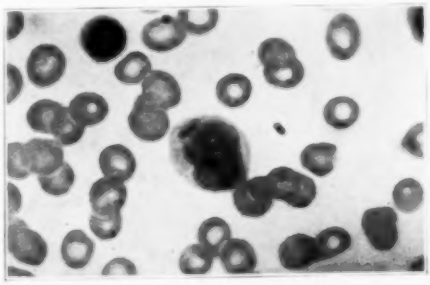


Fig. 1.—Blood smear. The large cell in the center is a myeloblast with an Auer body in its cytoplasm.

interpretation of the present case to consider in more detail the morphology of the cells as they appeared in the peripheral blood and in tissue sections.

In blood smears the cells were large, varying in diameter from 11 to 18 microns. The nucleus was large, usually occupying the greater part of the cell. In most cases the nuclear outline was irregular, there being one or more indentations, or the nucleus had an irregularly lobulated appearance, corresponding to cells of the Rieder type (Fig. 1). In preparations stained with Wright's or Jenner-Giemsa stains, the nucleus contained a very fine meshed purplish network, in which there were no large clumps of chromatin. These cells differed from lymphocytes in the relative homogeniety and fine, thin skeined, almost granular

17. Gorham, L. W.: Acute Myelogenous Leukemia, Albany M. Ann. **38**: 201, 1917.

structure of their nuclei. Several distinct clear areas, usually referred to as nucleoli, were frequently present in the nucleus. The cytoplasm was basophilic, and usually had a lighter perinuclear zone or an area of lighter cytoplasm at the nuclear indentation. In these clear areas, and to a less extent throughout the cytoplasm, were varying numbers of azure granules, usually only a few. Auer bodies were seen in the cytoplasm of some of these cells. Except for the irregularity of their nuclei, the cells closely resembled the "lymphoidocyte" as depicted by Pappenheim.[18] With the oxidase stain, the azure granules and Auer bodies reacted positively.

In tissue sections the cells were equally characteristic. The nuclei presented the same irregularities in outline that were seen in the peripheral blood, although cells with round or oval nuclei were also present. The nuclei were clear, the nuclear membranes distinct, and a few

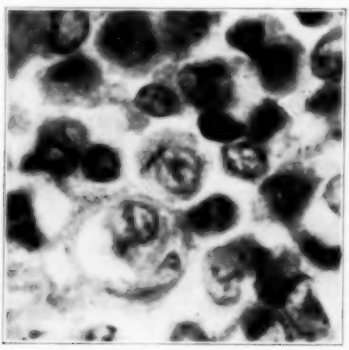


Fig. 2.—Section of splenic pulp, stained with azure II-eosin. The central cell contains an Auer body.

small chromatin particles were scattered throughout. One or two basic nucleoli, and in preparations stained with Goodpasture's stain, a distinct reddish nucleolar-like structure were seen in most of the cells. The cytoplasm was lavender, and sometimes contained an Auer body (Fig. 2). These cells were easily distinguished from the lymphocytes by their clear, irregular nuclei, relatively devoid of chromatic material, and their distinctly less basophilic cytoplasm. It was equally easy to distinguish them from "stem cells," which have deeply basophilic (azurophilic) cytoplasm and large, clear, regular nuclei.

With the oxidase reaction, the myeloblasts showed large numbers of granules, and the Auer bodies, reacting positively, were rendered much more distinct (Fig. 3).

---

18. Pappenheim: Hematologische Bestimmungstafeln. Leipzig, Werner Klinkhardt, 1920.

From the above, it is apparent that the cells were not typical of any one type. In smears they differed from Pappenheim's "lymphoidocyte" only in the irregularity of the nucleus; from the monocytes, in the fineness of the chromatin network, the presence of several distinct nucleoli, and a perinuclear zone of lighter cytoplasm. In sections they differed from "stem cells" in the less basophilic character of their cytoplasm and the irregularity of the nucleus.

I believe that they were cells in the early stages of myeloid differentiation. Inasmuch as definite granules could not be demonstrated without the oxidase reaction (pathologic retardation?), they were not

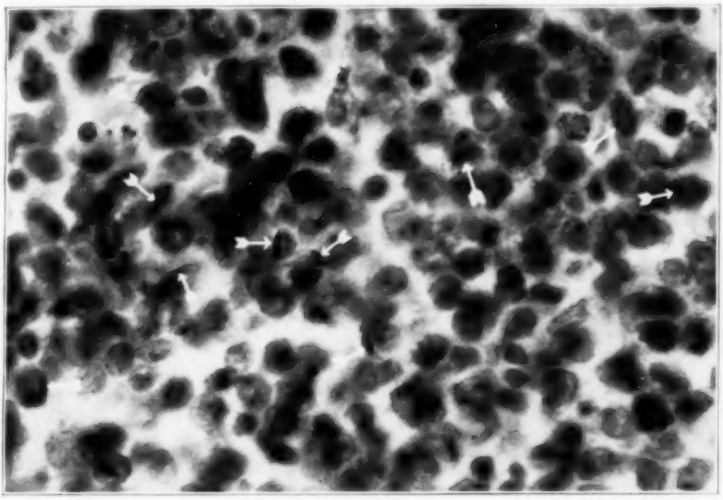


Fig. 3.—Section of splenic pulp, stained with oxidase stain. Some of the more prominent Auer bodies are indicated by arrows.

myelocytes, and are referred to in this paper as "myeloblasts," although it is realized that they were not identical with the "myeloblasts" of Naegeli.

The Auer Bodies.—The Auer bodies in the present case did not differ from those previously reported. They were distinctly seen in fresh blood preparations as refractive rod-shaped bodies. In smears stained with Wright's stain, they appeared bright red. Jenner's stain did not stain them. They varied in length from 1 to 5.2 microns, and in width from very fine lines to 0.75 microns. The rods were not always of the same form, sometimes being thicker at one end than the other, or thicker in the middle than at either end. The ends were

sometimes rounded, sometimes pointed, sometimes square, the two ends not always being alike. Usually there was only one Auer body in a cell, occasionally two or three.

The Auer bodies were always in the cytoplasm, usually, but not always, tangential to the nucleus, and frequently lay in the deep nuclear indentation. Sometimes they were over or partially over the nucleus, but not within it. They did not lie within vacuoles. Occasionally a rod was seen in the vicinity of a disintegrated cell, and once in a myelocyte, but in no other instance were they free in the blood plasm or in any cells other than myeloblasts.

In tissue sections, the Auer bodies appeared about the same as in blood smears, i. e., they were of the same form and position. By the methods with which they were stained, they appeared bright red. The bodies appeared a little larger on the average (up to 6.8 microns) in oxidase preparations than in the others. The measurements in sections were taken on rods lying in the plane of the section, to avoid error. With the oxidase stain, the bodies appeared of the same homogeneous structure as the other oxidase granules, and there seemed to be all gradations in size from the granules to the rods. In other than oxidase preparations, however, the granules were not to be seen, and the shorter rods were either faintly stained or not at all.

The demonstration of Auer bodies in tissue sections has not thrown much light on their nature or origin. It does, of course, add another argument against their being artefacts. A body demonstrable in preparations of so many different kinds—fresh blood, blood smears and tissue sections—and by different stains and technical methods, can hardly be considered as such. Furthermore, they probably occur in only one type of cell (myeloblasts), only in one part of the cell (cytoplasm), and possibly in only one disease (acute leukemia)—this last, of course, if Roth's case and the present one may be so interpreted.

Ishikawa's investigations with bacteriologic stains and methods practically exclude them from the class of bacteria, and the absence of detailed structure, especially nuclear material, is against the possibility that they are parasites of some other type.

Special mitochondrial methods were not used. There are important differences, however, between mitochondria and Auer bodies, both morphologically and in their staining reactions. Most of the stains which are useful in demonstrating the Auer bodies contain azure, which is a poor mitochondrial stain.[19] Wallin [20] also found mitochondria stainable by pyronin-methyl green, which, according to Pappenheim, does not stain Auer bodies.

---

19. One azure containing mixture (Giemsa's stain) has recently been found by Cowdry and Olitsky to stain mitochondria little, if at all (J. Exper. M. **36**: 521, 1922).

20. Wallin, I. E.: On the Nature of Mitochondria. III. The Demonstration of Mitochondria by Bacteriologic Methods. Am. J. Anat. **30**:451, 1922.

That they are related to cell granules [21] is suggested by the presence of bodies of all intermediate sizes. Of these, their staining reaction most closely resembles granules of the methylene-azure group. In tissue sections, however, they are demonstrable by methods that do not stain the azure granules.[22]

The globular bodies in the spleen and lymph node probably do not belong to the same class of substances as the Auer bodies. With Dominici's stain, they were purple instead of red, and appeared in plasma or lymphoid cells instead of in myeloblasts. Resembling, as they did, the "mulberry cells" of Weber, or the "fuchsin bodies" of Russell, it is more probable that they were some form of hyalin (Weber and Blendinger,[16] Goodpasture [23]). Furthermore, as previously shown, the fuchsin bodies were strongly gram-positive (Russell,[24] McConnell and Lang [25]) while the Auer bodies were not demonstrable by the Gram stain (Ishikawa).

SUMMARY

1. Auer bodies have been demonstrated in microscopic sections of a case that was probably one of acute myelogenous leukemia.

2. The bodies are demonstrable in paraffin, celloidin and frozen sections, and by several stains.

3. In the oxidase stain they react positively, both in blood smears and in tissue sections.

4. Wright's blood stain, Giemsa's and Goodpasture's acid polychrome-methylene blue and eosin are very satisfactory for their demonstration. Dominici's stain and azure II-eosin stain them, but less distinctly. They were not stained by hematoxylin and eosin, Gabbett's stain, nor by Gram's method.

5. Auer bodies have the same morphologic appearance in tissue sections that they have in blood smears.

6. The Auer bodies were found within the cytoplasm of atypical myeloblasts, and, in blood smears, in the immediate vicinity of disintegrated cells. Only once was an Auer body seen in a myelocyte.

7. The Auer bodies are not identical in staining reactions with any of the known normal cell granulations, nor with the hyaline bodies (Russell's fuchsin bodies) which were present in the same sections.

---

21. Elongated granules (rod or spindle shaped) occur normally in some of the lower vertebrates, as birds and reptiles.

22. Azure granules were not seen in tissue sections except with the oxidase method, although Auer bodies were stained by other methods as well.

23. Goodpasture: Crystalline Hyalin., J. M. Research **35**:259, 1917.

24. Russell, William: An Address on a Characteristic Organism of Cancer, Brit. M. J. **2**:1356, 1890.

25. McConnell and Lang: Russell's Fuchsin Bodies, J. M. Research **42**:99, 1920.

THE EFFECT OF SPLENECTOMY ON THE HEMOPOIETIC SYSTEM OF MACACUS RHESUS *

E. B KRUMBHAAR, M.D., AND J. H. MUSSER, JR., M.D.
PHILADELPHIA

The diversity of the reported results of splenectomy in different species of animals has been a striking feature in publications on this subject,[1] and has contributed to a rather widely held view that comparatively little is known in regard to the functions of the spleen. Nowhere is this fluctuation of opinions and prevalent agnosticism better shown than in the editorials of the *Journal of the American Medical Association*.[2] Recognizing that much still remains to be learned about the various functions of this complex organ, and (as is usually the case when advances are being made in any given field) that not a few observations by different investigators are at variance, nevertheless, it should be recognized that material progress has been made, and in the relation of the spleen to blood formation and blood destruction, at least, a number of positive facts can be accepted, as we have tried to show elsewhere.[1]

These facts have chiefly been elicited by studying the structural and functional changes produced by the removal of the spleen either from normal or experimentally altered animals, and of administering hemolytic agents to animals that have had their spleens removed, ligated, or diverted from the circulation by a modified Eck fistula. The temporary anemia and leukocyte changes, the increased resistance of the erythrocytes to hypotonic salt solution, the lessened tendency of the organism to the production of hemoglobinuria and jaundice, the compensatory changes in the liver, lymph nodes and bone marrow, and the importance of the circulatory factor in bringing about these changes, are all items that have previously been commented on and that need not be more than mentioned at this moment.

Although our findings in the dog agreed fairly closely with the available data on man, nevertheless, they differed in several particulars from the results found by other investigators in the rabbit, cat and

---

* From the John Herr Musser Department of Research Medicine of the University of Pennsylvania and the Laboratories of the Philadelphia General Hospital.

* The manuscript and slides have been placed in the National Depository at the Army Medical Museum, Washington, D. C.

1. Pearce, R. M.; Krumbhaar, E. B., and Frazier, C. H.: The Spleen and Anemia, Philadelphia, J. B. Lippincott Company, 1918.

2. J. A. M. A. **59**:1546 (Oct. 25) 1912; **77**:1577 (Nov. 12) 1921; **77**:1823 (Dec. 3) 1921; **78**:1056 (April 8) 1922.

guinea-pig. We, therefore, have thought it advisable to study some of these changes in the monkey with the idea not only of obtaining comparative data in yet another species, but also that the results obtained from an animal more nearly related to man might give a more reliable basis for comparison with the study of the function of the human spleen.

METHODS

The blood pictures of six monkeys (Nos. 1, 2, 5, 6, 10 and 12) were followed over periods varying from six weeks to two years after splenectomy, with two nodectomized monkeys as control. The response to a hemolytic agent was observed in four splenectomized monkeys and four controls, and the hemopoietic system of eight monkeys and controls studied post mortem. The following items of the blood picture were studied: hemoglobin,[3] erythrocytes, leukocytes, differential counts, reticulated erythrocytes, platelets and resistance of erythrocytes to hypotonic salt solution. On account of numerous accessory factors that may influence the blood picture, such as age, feeding, emotion, exercise, etc., especial care was taken to standardize conditions as far as possible by having counts made at the same time of day, at the same interval after feeding, by the same operator, and with the same instrument, and using only healthy animals of as nearly the same age and size as the control as could be obtained. At this point it should be noted that size and weight are not indications of age in this species, as we have had very small monkeys with correspondingly "young" teeth remain relatively unchanged in size and weight over periods of more than three years. Two or more preliminary counts were made over periods varying between a week and three months before beginning the experiment. In spite of these precautions, variations were occasionally obtained so much greater than the limits of error of the methods employed, that they must be attributed to other unknown factors not controlled or to individual differences in response. Unlike the domestic animals previously used, most of the monkeys tended to lose some weight under laboratory conditions, but this loss proved no greater in the test animals than in the normal controls, and was surprisingly independent of the severity of the anemia. Tuberculosis did not prove to be a disturbing factor.

3. Hemoglobin estimations were begun with a Sahli instrument that happened to check closely with simultaneous determination of the oxygen capacity of the blood. Later the Newcomer method was also used, but for the sake of uniformity only the Sahli figures are given. Though the Newcomer glass was also checked with the oxygen capacity method it tended to give lower readings, and differences between the two methods fluctuated for reasons that we have been unable to ascertain.

RESPONSE TO SPLENECTOMY

The results of simple splenectomy we have found to be less marked in the monkey than in either man or dog, but of the same nature. A moderate anemia [4] began almost immediately after operation, although on the first day or two a slight rise in the blood picture was noted in two cases. While this rise is hardly beyond the limit of accuracy of

TABLE 1.—ERYTHROCYTE COUNTS AFTER SPLENECTOMY (IN MILLIONS)

	Monkey 1	Monkey 2	Monkey 5	Monkey 6	Monkey 10	Monkey 12	Monkey 11
Relative spleen and body weight	0.09%	0.09%	0.14%	0.10%	0.18%	0.08%	Control Nodectomy
Before splenectomy	6.40	7.14	6.89	6.14	5.60	5.14	5.86
Before splenectomy	5.90	....	5.90	6.03	5.20	4.90	5.76
1 day after	5.49	5.89	5.93	5.88	5.50	5.29	5.84
3 days after	....	....	**4.10**	5.53	**4.43**	4.88	5.96
5 days after	....	....	....	4.91	....	5.04	5.55
7 days after	5.56	6.12	4.55	4.89	4.80	**4.75**	5.78
9 days after	....	....	....	**4.69**	4.48	5.02	5.68
14 days after	**5.17**	**5.20**	5.09	....	4.95	5.39	5.56
21 days after	6.03	5.82	5.51	5.09	....	5.36	....
28 days after	6.10	5.41	5.55	5.09	....	5.25	....
35 days after	6.58	5.55	....	5.13	....	5.52	....
42 days after	6.37	5.94	5.60	5.40	....	6.34	....
2 months after	6.10	5.50	6.06	6.23	5.16	5.56	....
3 months after	....	....	5.89	6.10	....	....	....
4 months after	6.50	4.90	6.19	6.16	....	....	....
12 months after	Killed	Killed	6.04	5.90	6.35	6.4	....
15 months after	....	....	6.40	6.26	6.30	6.0	6.00
24 months after	....	....	6.44	6.02	6.16	6.16	6.51

TABLE 2.—HEMOGLOBIN ESTIMATIONS AFTER SPLENECTOMY

	Monkey 1	Monkey 2	Monkey 5	Monkey 6	Monkey 10	Monkey 12	Monkey 11
Before splenectomy	98	102	92	98	102	97	90
Before splenectomy	94	...	85	92	98	97	92
1 day after	83	86	75	88	105	88	94
3 days after	...	...	**71**	86	**86**	86	92
5 days after	...	...	...	82	...	90	90
7 days after	91	85	78	86	94	**84**	95
9 days after	...	...	...	85	88	86	102
14 days after	**73**	**83**	85	...	100	96	86
21 days after	86	93	90	**81**	...	98	...
28 days after	100	94	93	85	...	88	...
35 days after	102	96	...	92	...	100	...
42 days after	103	101	103	98	...	102	...
2 months after	99	97	99	100	...	96	...
3 months after	...	...	97	101	100	...	...
4 months after	100	83	102	96	...	...	...
12 months after	Killed	Killed	93	86	...	96	...
15 months after	...	...	103	92	91	91	92
24 months after	...	...	108	92	88	92	98

the method, we think it is probably to be explained by some such factor as a greater concentration due to postoperative change in blood volume. The greatest degree of anemia, which, however, never reached a point lower than four million cells per cubic millimeter and 71 per

4. For the blood picture of normal monkeys under our laboratory conditions see J. M. Research, **42**:105, 1921.

cent. hemoglobin, was reached in the first two weeks and the return to normal was accomplished before the second month. There was no subsequent noteworthy polycythemia, such as was reported in some of the early dogs studied. No essential difference was noted between the hemoglobin and erythrocyte curves. As controls, two monkeys were used, on which laparotomies were performed, the spleens handled, and an abdominal lymph node removed. From the example given in the table, it will be seen that no anemia resulted in spite of these procedures and of frequent taking of blood samples. Estimations of blood volume by the vital red method of Keith, Rowntree and Geraghty in the postsplenectomy anemia of dogs show that there are actually fewer corpuscles in the vascular system and not merely a lessened concentra-

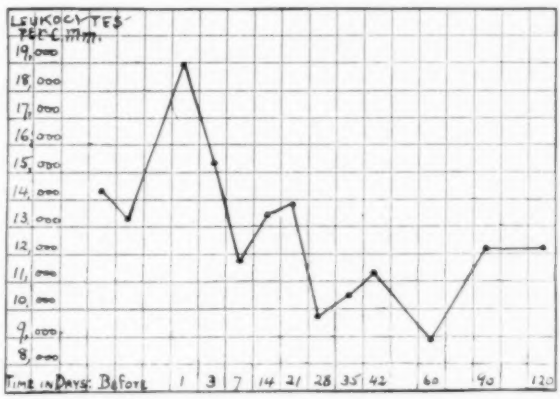


Chart 1.—Leukocyte count in splenectomized monkeys. Composite curve based on six test monkeys.

tion due to hydremia. It did not prove feasible to carry out similar tests on the monkeys of this series.

The resistance of the erythrocytes (which before splenectomy averaged complete hemolysis at 0.33 per cent. sodium chlorid and beginning hemolysis at 0.45 per cent.) was increased in every splenectomized animal. This was usually evident on the first or third day after operation and continued during the period of observation. Averages in six splenectomized monkeys showed that the increase was maintained during the period of observation and reached a maximum complete hemolysis at 0.26 per cent. and beginning hemolysis at 0.42 per cent. This striking increased resistance of the erythrocytes, persisting over such a long period, confirms our previously expressed view that it constitutes one of the most important results of splenectomy. Its occurrence in disease

is probably in the same way one of the chief beneficial factors in those primary blood diseases that are helped by splenectomy.[5]

That the anemia is due to lessened blood formation is indicated by the absence of normoblasts and Howell-Jolly bodies in the blood smear and almost complete disappearance of reticulated erythrocites, which, however, are usually not more than 0.1 per cent. in the normal monkey. The changes in the platelet counts were inconstant and never beyond the wide variations that are usually found in counting these elements

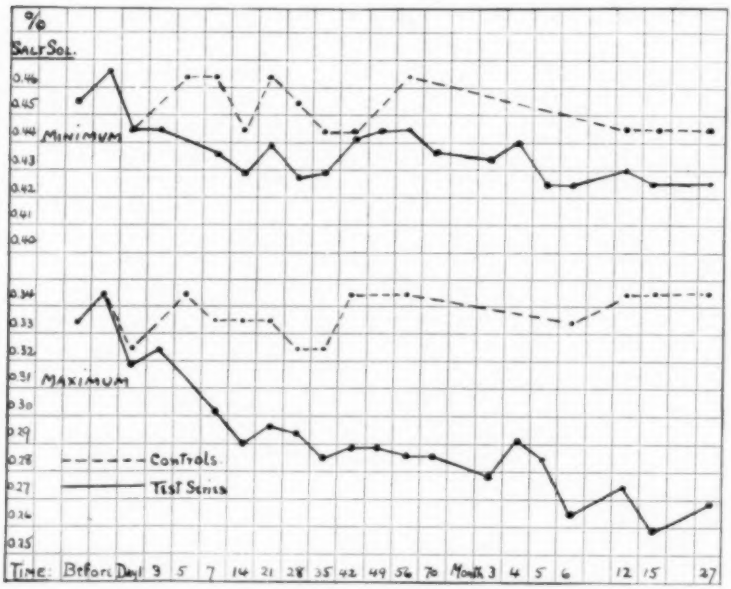


Chart 2.—Resistance of erythrocytes of splenectomized monkeys. Composite curve based on estimations on six test monkeys and two controls. Upper solid line, minimum resistance of test monkeys; dotted line, controls. Lower solid line, maximum resistance of test monkeys; dotted line, controls.

under normal conditions. At any rate, there were no signs of a "blood crisis," such as is frequently observed after removal of the diseased spleen of man. We have previously suggested that the anemia may be due to a loss with the spleen of a substance having a stimulating action on the erythrogenetic centers of the bone marrow, but we have no further evidence at present to support this view. The urobilin

5. A discussion of the various views as to the cause of this increased resistance will be found on page 50 et seq. of our book on the spleen and anemia.[1]

excreted in the feces, as estimated by the Wilbur and Addis method, was so small that it was obviously impossible for splenectomy to produce any changes that would be beyond the limits of error of this method, and we, therefore, were without quantitative criteria of the amount of blood destruction.

The total leukocyte count of the monkey, which in our series, averaged 14,200 per c. mm. before splenectomy was only raised a few thousand by the operation (to an average of 19,300 per c. mm.), and by the end of a week had fallen below the normal level. This is in marked contrast to the greater and more persistent rise that occurs in man and in the dog. The differential count, however, showed constantly both a relative and absolute increase in the number of polymorphonuclears and a decrease in the number of lymphocytes, a condition that returned approximately to normal in from two to three months. In the counts made more than a year after splenectomy, the lymphocytes were definitely increased. Taken with the increase in size and number of lymph nodes that we have observed in the splenectomized monkey post mortem this would indicate that the lymphoid rôle of the spleen is relatively more important and its erythrolytic function less important in the monkey than in the dog and man.

RESPONSE TO HEMOLYTIC AGENT

As a hemolytic agent, toluylendiamine was first tried in doses equivalent to those previously used successfully with dogs. The insignificant amount of hemolysis produced confirms the experience of other investigators that different species show considerable variations in resistance to this drug. Sodium oleate was next given to two groups of monkeys, each group made up of a monkey anemic after splenectomy, one that had recovered from his anemia and a normal control. What proved with three monkeys to be a lethal dose (Nos. 1, 2 and 4, 0.2 gm. per kilo injected intravenously over a period of fifteen minutes) in others only caused hemoglobinuria for a few hours, without subsequent choluria, and produced a mild anemia that lasted about two weeks. An increase of both maximal and minimal resistance of the erythrocytes, amounting to from 0.4 to 0.8 per cent. sodium chlorid, was noted in all but one monkey receiving sodium oleate. As most of these had recently been splenectomized, however, no deductions can be made from this observation. In this group, the anemic splenectomized monkey (No. 10) showed the greatest loss of blood and longest period of repair, but the nonanemic splenectomized monkey (No. 5) whose erythrocytes were the most resistant of the three, showed even less effects from the oleate than the normal control (No. 14). In the other group (receiving 0.15 gm. per kilo of sodium oleate)

the nonanemic splenectomized monkey, whose erythrocytes were also the most resistant, showed the most marked anemia and the longest period of repair. Although it is obvious that no conclusions can be drawn from such discordant results, they are recorded as instances of the complexity of the problem, with the suggestion that possibly the variations may be brought about by the two factors (loss of spleen and resistance of erythrocytes) operating to different degrees in different

TABLE 3.—Response to Hemolytic Agent; 0.2 Gm. Sodium Oleate Being Given Intravenously

Resistance to Salt Solution	Monkey 5: Splenectomy, 7 Weeks; C. H. : 0.36; B. H. : 0.42		Monkey 10: Splenectomy, 2 Weeks; C. H. : 0.30; B. H. : 0.42		Monkey 14: Normal; C. H. : 0.34; B. H. : 0.46	
	R. B. C.	Hgb.	R. B. C.	Hgb.	R. B. C.	Hgb.
Before sodium oleate	5.5	85	5.0	80	5.6	78
1 day after	6.2	**76**	3.9	67	**4.9**	**72**
2 days after	**5.6**	80	**3.7**	**59**	4.9	76
4 days after	5.8	88	4.4	70	5.3	75
6 days after	5.8	81	4.5	81	5.1	82
9 days after	6.0	96	4.6	80	5.5	84
11 days after	6.6	...	5.0	84	5.3	84
14 days after	5.9	81	4.4	88	5.4	84
16 days after	5.7	81	4.2	82	5.4	86
Greatest drop	...	9	1.3	21	0.7	6

TABLE 4.—Response to Hemolytic Agent; 0.15 Gm. Sodium Oleate Being Given Intravenously

Resistance to Salt Solution	Monkey 1: Splenectomy, 17 Weeks; C. H. : 0.28; B. H. : 0.46		Monkey 6: Splenectomy, 9 Days; C. H. : 0.34; B. H. : 0.44		Monkey 11: Normal; C. H. : 0.32; B. H. : 0.44	
	R. B. C.	Hgb.	R. B. C.	Hgb.	R. B. C.	Hgb.
Before sodium oleate	6.5	100	4.7	85	5.5	95
1 day after	6.34	100	5.0	78	5.6	90
2 days after	6.3	88	4.6	74	5.7	94
4 days after	**4.8**	**88**	**4.2**	**72**	**4.9**	**86**
6 days after	5.2	90	4.6	75	4.9	86
9 days after	4.8	90	4.9	79	5.3	92
13 days after	5.0	96	5.1	81	5.4	90
15 days after	5.0	94	5.4	89	5.5	95
21 days after	5.7	94	5.1	92	5.6	94
Greatest drop	1.54	12	0.5	13	0.6	9

subjects. It is apparent from these experiments that the toxicity of sodium oleate for monkeys is too great to permit sufficient latitude for its profitable use as a hemolytic agent.

PATHOLOGY

Opportunity to study changes in the viscera after splenectomy was offered in five monkeys (Nos. 1, 2, 5, 6 and 10) with three others as controls (Nos. 4, 8 and 13). The high cost of monkeys since the war prevented the others from being sacrificed for this purpose. In

two of the five (Nos. 1 and 5) biopsy specimens of bone marrow had been removed, one at the same time as the spleen, the other ten weeks after splenectomy, and in two other cases (Nos. 11 and 12) abdominal lymph nodes were removed as operative controls. Of the five splenectomized monkeys, two monkeys (Nos. 1 and 2) were killed by overdoses of sodium oleate five and four months, respectively, after splenectomy, and the third monkey was sacrificed two years after splenectomy, having been normal for a long period. For both the oleate monkeys death came suddenly while the injection was in progress, from causes that we could not establish, but certainly not from excessive blood destruction. Except for a few erythrocyte bearing phagocytes

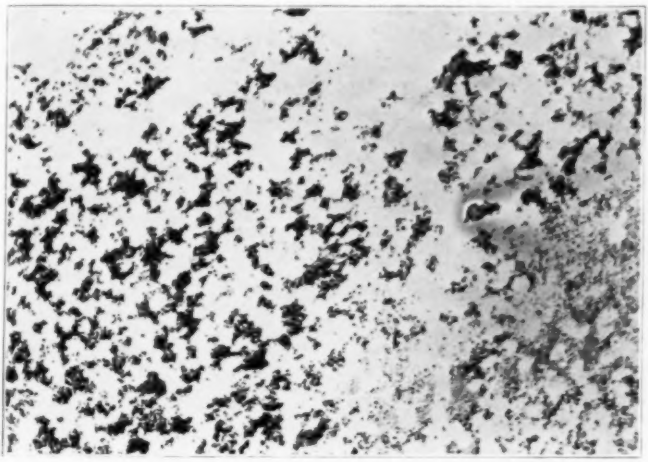


Fig. 1.—Bone marrow (Monkey 1), removed at biopsy, ten weeks after splenectomy. × 65.

in one lymph node, the post-mortem findings were those that we have customarily found after uncomplicated splenectomy. One monkey (No. 5), though normal during life, at the necropsy was found to have the bone marrow studded with lymphoid follicles, an observation that has been reported by one of us elsewhere.[6] One monkey (No. 10) was chloroformed while in good health, twenty-seven months after splenectomy. We shall consider these four animals together, with the reservations that the above facts necessitate. One monkey (No. 6) died of generalized tuberculosis, apparently developing sometime after the conclusion of the experimental work, thus becoming useless for this study, and one monkey (No. 12) still lives.

6. J. M. Research, **43**:369, 1922.

Bone Marrow.—In the bone marrow removed coincident with the spleen of the control monkeys, a pink or even splotched reddish appearance was found by histologic examination always to be due to more or less congestion, the hemopoietic cellular content being slight, and never filling more than from 10 to 40 per cent. of any given area. Estimation of the amount of bone marrow hyperplasia is made very difficult by the great variation that occurs often in closely adjacent areas. An attempt was made to cover this difficulty by considering the extremes as well as the general appearance. Definite leukogenetic and erythrogenetic islands in Bunting's sense were impossible to distinguish, though

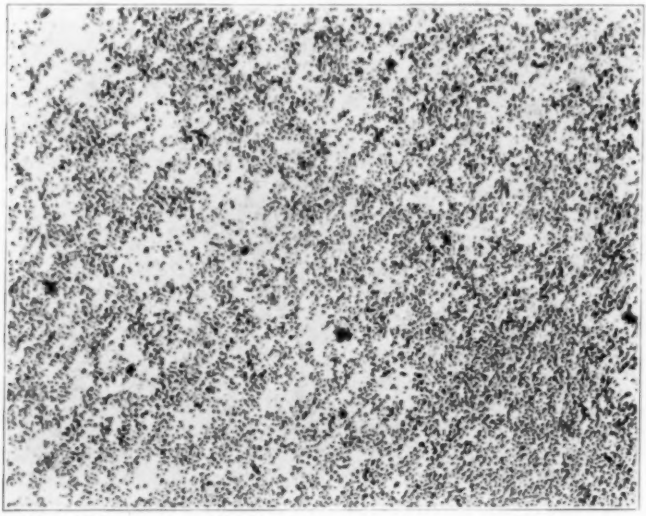


Fig. 2.—Bone marrow (Monkey 1), at necropsy, four and a half months after splenectomy. ×92.

there was, at times, distinct clumping of the normoblasts. In the bioptic bone-marrow of monkey 5, the polymorphonuclears and normoblasts were especially prominent, with the other customary bone marrow cells approximately in their usual proportions. A small amount of hemosiderin pigment was found contained within phagocytes in about the same amount as is found in normal spleens. No mitosis was found.

In the bioptic bone marrow of the monkey splenectomized after ten weeks (No. 1), the cellular areas never exceeded 20 per cent. of the field. Hemopoietic cells were found in the following proportions: Mononuclear cells (primordial cells, myeloblasts, myelocytes, histiocytes [?]), 55 per cent.; polymorphonuclears, 28 per cent.; normo-

blasts, 12 per cent.; megaloblasts, 2 per cent.; eosinophil myelocytes, 1 per cent.; eosinphil leukocytes, 1 per cent.; giant cells, 1 per cent.; in other words a predominance of immature leukogenetic and possibly of nonhemopoietic cells.

At necropsy, the bone marrow hyperplasia of the splenectomized animals was found to be always present and distinctly greater than the controls, from 70 to 95 per cent. of large areas being filled with hemopoietic cells. For instance, in monkey 1, killed four and one-half months after splenectomy, the bone marrow was a deep reddish pink and cells occupied more than 95 per cent. of many areas, in the follow-

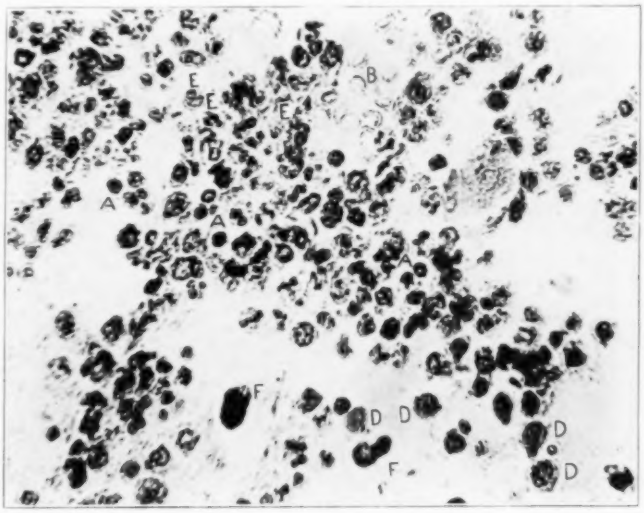


Fig. 3.—Bone marrow (Monkey 1), oil immersion, ×600. Note a, normoblasts; b, erythrocytes; d, myelocytes; e, polymorphonuclears; f, phagocytes with pigment, etc.

ing proportions: Mononuclear cells, 24 per cent.; polymorphonuclears, 45 per cent.; normoblasts, 22 per cent.; eosinophilic myelocytes, 2 per cent.; eosinophilic leukocytes, 2 per cent.; lymphocytes (?), 1 per cent.; mononuclear giant cells, 1 per cent.; multinuclear giant cells, 2 per cent.; megaloblasts, 1 per cent. The site of the previous operation was filled in with new bone, the cavity being filled with orange colored gelatinous material and loose bone spicules. The marrow of monkey 2, killed four months after splenectomy, showed a somewhat larger percentage of polymorphonuclears, and greater variations in the cellular content of different areas. In the other two monkeys (Nos. 5 and 10) the hyperplasia was not so marked, being about 80 and 70 per cent. in

most fields, but in a few areas reaching as low as 40 and 10 per cent., respectively. This decrease was apparently chiefly due to a lesser number of polymorphonuclears. In the control bone marrows the cellular areas occupied from 10 to 40 per cent. of the whole, with a larger number of immature forms. For instance, the bone marrow of monkey 8 (cause of death undetermined), though deeply congested like the other organs, microscopically was from 10 to 40 per cent. cellular, in these proportions: mononuclear cells, 66 per cent.; polymorphonuclears, 10 per cent; normoblasts, 20 per cent; eosinophil myelocytes, 1 per cent.; eosinophil leukocytes, 1 per cent.; giant cells, 2 per cent. Mitosis was only found a few times in the marrow of one of the

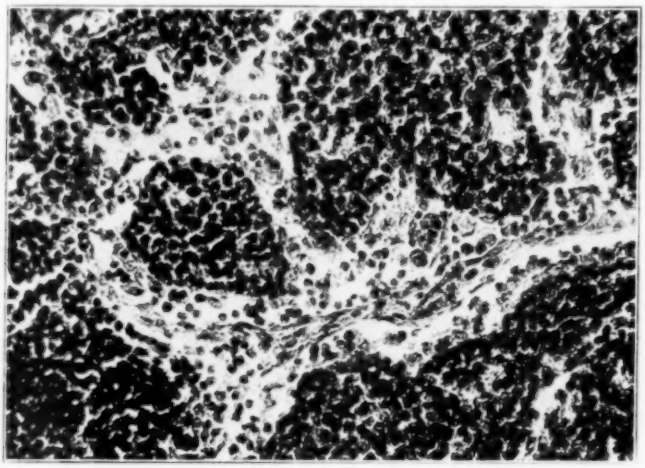


Fig. 4.—Lymph node (Monkey 1), at necropsy, four and a half months after splenectomy. × 276. Note the sinuses moderately filled with phagocytes, many of which contain hemosiderin pigment.

splenectomized animals. Hemosiderin pigment—both intracellular and extracellular—was found somewhat more commonly than in the controls.

These results confirm the findings of Pearce and Pepper,[7] with the difference that in these long time splenectomies there was no exception to the development of cellular hyperplasia. Leukogenesis and erythrogenesis may both be said to have been active, though to different degrees in the different animals. On the whole, myelocytes and undifferentiated mononuclear forms were relatively more frequent in the controls than in the splenectomized animals. It should be borne in mind, also, that

7. Pearce, R. M., and Pepper, O. H. P.: The Changes in the Bone Marrow After Splenectomy, J. Exper. M. **20**:19, 1914.

there is no definite criterion for some of the hemopoietic cells and many in the hyperplastic areas may have been concerned, not with hemopoiesis, but with the utilization of iron or other activities, taken over in the absence of the spleen.

Lymph Nodes and Kupffer Cells of Liver.—The mesenteric, gastrohepatic and prevertebral lymph nodes of five splenectomized and four control monkeys were studied at necropsy, and in three cases nodes obtained at biopsy were also available. In two cases, nodes from all parts of the body were examined, without obtaining any additional information; in one case tuberculosis and in two cases camphorated oil lesions masked the picture. In all the splenectomized monkeys the visceral lymph nodes seemed abnormally prominent; and on microscopic

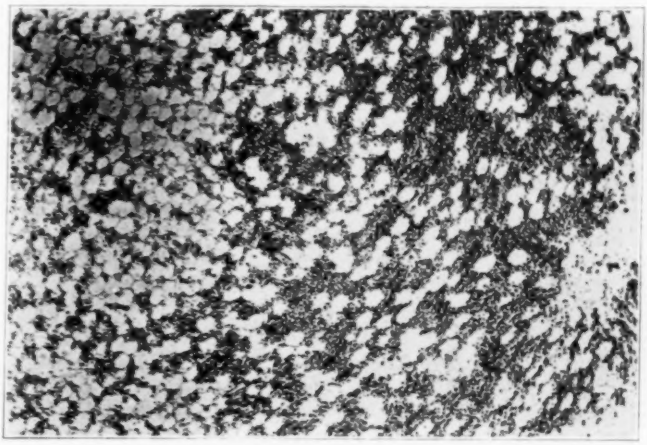


Fig. 5.—Bone marrow (Monkey 2), animal killed four months after splenectomy. × 69.

examination the large mononuclear cells of the sinuses (endothelial leukocytes, macrophages, etc.) were distinctly increased over the normal, although the amount of pigment contained in them was no greater than was sometimes found in presumably normal nodes of the controls (No. 4). Neither was any phagocytosis of erythrocytes observed, nor would such be expected in the absence of any acute, severe hemolytic process. In spite of the most careful search, no hemolymph nodes could be found. In a few cases, nodes were found that were dark red at one end, but this was always found on microscopic examination to be due to congested vessels within the follicles or sinuses, and at most only a few scattered erythrocytes were free in the lymph sinuses. In the two monkeys (Nos. 1 and 2) that died during the administration

of sodium oleate, the macrophages were markedly increased, and a considerable number contained hemosiderin pigment or showed a cytoplasm of a peculiar coppery appearance, but never contained erythrocytes, so that the causative action of the oleate is problematical.

The stellate endothelial cells (Kupffer cells) of the liver likewise seemed to be more numerous and prominent in the splenectomized monkeys than in the controls. They contained more or less hemosiderin pigment (as did one of the controls, No. 4); but in no case was this extreme or were erythrocytes found within the phagocytes. In one monkey (No. 10) hemosiderin, and probably hemofuscin, were also found scattered as fine granules through the liver cells. A similar picture has been reported in hypertransfused rabbits.[8] Examination of other organs revealed nothing pertinent to this study.

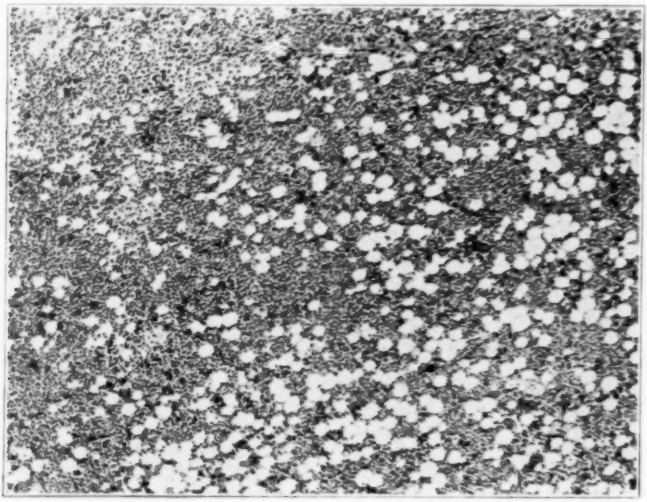


Fig. 6.—Bone marrow (Monkey 10), animal killed two years after splenectomy. ×69. Note the very marked cellular hyperplasia.

These findings, both in lymph nodes and in the Kupffer cells, are in accord with Pearce and Austin's previous studies[9] on dogs and confirm their suggestion that in splenectomized animals these organs, together with the bone marrow, take over, or at least develop to a greater extent, the part played by the spleen in the disposition of effete erythrocytes and there disintegration products.

8. Krumbhaar, E. B., and Chanutin, A.: Studies in Artificial Plethora, J. Exper. M. **35**:847, 1922.

9. Pearce, R. M., and Austin, J. H.: Changes in the Endothelial Cells of the Lymph Nodes and Liver of Splenectomized Animals Receiving Hemolytic Serum, J. Exper. M. **16**:780, 1912.

DISCUSSION

It is obvious that while the results of removing the monkey's spleen are less marked than in the case of dog or man, they tend in the same direction, as opposed to the negative findings in rabbit and guinea-pig. In searching for possible explanation of these differences, the relation of the weight of the spleen to the body weight was compared in the different species. In twenty-three normal dogs, we have found that the spleen averaged 0.25 per cent. of the body weight, and in seven normal monkeys the spleen averaged 0.13 per cent., with variations between 0.09 and 0.18 per cent. Figures given by Welcker and Brandt [10] for the other species are: man, 0.25 per cent.; guinea-pig, 0.13 per cent.; rabbit, 0.05 per cent. It would, therefore, be logical to expect that splenectomy would produce less results in the species with the relatively smaller spleens, especially as it is believed that the spleen is only one of several elements in the hemopoietic system, and that other elements may acquire the ability to compensate for its absence. In our monkeys, the most marked change occurred in those that had had relatively larger spleens, though it must also be recognized that the initial resistance of the erythrocytes was also a factor. This hypothesis is further supported by Wolferth's [11] observations that the same changes occur in the albino rat after splenectomy that we found in dogs [12] and that in eight rats with abnormally large spleens the changes were even more marked and proved fatal in seven.

RESULTS

1. In the *Macacus Rhesus,* splenectomy produces an anemia that is much less marked than in the case of dog or man. The resistance of the erythrocytes is increased throughout the period of observation.

2. The number of reticulated erythrocytes is diminished and no signs of a "blood crisis" are to be found.

3. The total leukocyte count is only slightly increased during the first week, but an absolute and relative increase of polymorphonuclears and decrease of small lymphocytes persists for a longer period.

4. The *Macacus Rhesus* is resistant to toluylenediamine hemolysis. Sodium oleate causes an anemia that may be more severe and last longer in the splenectomized animal, but greater resistance of the erythrocytes may apparently outweigh the loss of the spleen.

5. The bone marrow is very little, if at all, hyperplastic at early periods after splenectomy; but by the fifth month, cellular hyperplasia is marked and continues so to the latest period of observation (twenty-seven months).

---

10. Welcker, H., and Brandt, A.: Arch. f. Anthropol. **28**:37, 1902.
11. Wolferth, C. C.: Arch. Int. Med. **19**:105 (Jan.) 1917.
12. Relation of spleen to body weight of albino rat—0.25 per cent., according to Wistar Institute statistics.

6. The visceral lymph nodes are more prominent after splenectomy, and phagocytic action more marked. Hemolymph nodes were not found.

CONCLUSIONS

1. Therefore it appears that the transient postsplenectomy anemia results chiefly, or, perhaps, wholly from lessened blood formation: perhaps, due, as we have previously suggested, to the loss with the spleen of a substance which normally stimulates the bone marrow.

2. The persistent increased resistance of the erythrocytes is probably one of the most important results of splenectomy from a therapeutic point of view.

3. The histologic changes in the lymph nodes, bone marrow, and Kupffer cells of the liver of splenectomized animals indicate that these organs take over the spleen's share in disposing of effete erythrocytes and their disintegration products.

4. The different response of various animal species to splenectomy is at least partly explained by the difference in the relative spleen and body weights in the various species.

THORACOPLASTIC COMPRESSION OF THE LUNG IN PULMONARY TUBERCULOSIS*

PHILIP KING BROWN, M.D.
AND
LEO ELOESSER, M.D.
SAN FRANCISCO

I. MEDICAL ASPECTS (PHILIP KING BROWN)

There is but one therapeutic agency in the treatment of tuberculosis that has stood the test of all time, and that is rest. For many years the technic of rest has been studied and notable contributions to it have come into general use. A few are in the developmental stage and deserve study and trial in well selected cases. The five methods in addition to rest in bed which have been proposed for producing relative immobilization of all or part of one side of the lungs are: (1) (*a*) Postural rest, lying twenty-three and one-half hours a day on the affected side in one-sided cases, advocated by Webb. (*b*) Sand bags on the affected part (Sewell), giving, to less degree, the same result. (2) Section of the phrenic nerve in the neck on the affected side, causing paralysis of one half of the diaphragm and partial collapse of the lower lobe. (3) Local replacement by some foreign body of the area occupied by the affected part of the lung if in the apex, after extrapleural rib resection (Tuffier). (4) Collapse of the lung by nitrogen gas or air introduced into the pleural cavity under pressure (Forlanini, 1882; Murphy, 1898). (5) Resection of all the parts on one side, causing thereby a narrowing of the chest on the affected side commensurate with the collapse needed (Friedrich, Wilms, Sauerbruch, etc.).

The first method is so simple that it demands trial in every unilateral process. So great has been Webb's success with the method that it has largely replaced artificial pneumothorax in his hands. In his exhaustive article on the subject[1] he presents roentgen-ray plates showing how limited is the motion on the side on which the patient lies and the exaggerated excursion of the upper side. The object of the fifteen minute periods on the well side is to facilitate cavity drainage.

Of resection of the phrenic nerve it has been said by Morriston Davies that it is especially applicable when there are secondary changes, either mechanical or from infection to considerable extent in the lower

---

* From the Division of Surgery, Stanford University Medical School.
* Read before the Fifty-First Annual Meeting, Medical Society of the State of California, May, 1922.

1. Webb: Tr. National Tuberculosis Association, **12**:182, 1916.

lobe; when it is advisable to diminish the movement of the lower lobe when it is the seat of primary disease; to prevent aspiration of tuberculous or pyogenic material from the upper lobe cavity; and as a preventive measure against the development of bronchiectasis in chronic interstitial pneumonia.

Tuffier's procedure of partial thoracoplasty and introduction of foreign matter to aid compression, practiced also by Baer, has been used by numerous surgeons as an adjunct to extensive thoracoplasty, Archibald uses it in anterior apicoplasty, leaving the first rib intact, and feels that the shoulder is better supported in this way. Our own experience has been entirely with the efforts to bring about complete compression of the apex by removal of all or part of the first rib and shortening the clavicle.

There remain to be considered the two means of complete collapse of the affected side, artificial pneumothorax and thoracoplasty.

That pneumothorax has been a great contribution to the treatment of unilateral pulmonary tuberculosis, no one who has used it will deny. Like all therapeutic methods it had to go through a long experimental period, and doubtless its use has been abused badly. Too early cases in which there has been no trial of rest in bed in favorable surroundings, have undoubtedly been used to exploit the method. Too advanced cases offer only the palliative conditions following a lessened septic absorption. It is not a remedy to be used, except when the size of a cavity indicates the unlikelihood of closure if left to nature, although it is truly wonderful how much nature alone can do. A heart may be pulled over 2 or 3 inches, the diaphragm may retract several inches, the mediastinum contributes its bit by allowing considerable pushing over by the lung that is doing all the work, the shoulder on the affected side droops forward and in the roentgenogram the ribs are seen to be much closer together than on the good side. All this may not close a cavity or arrest activity, and even this much can only be accomplished when the pleuropulmonary fixation by adhesions assists the pulmonary fibrosis. Is it not obvious that anything which would assist the progress of fibrosis is to be encouraged? It is rarely the case that a lung in which retraction has gone on to a considerable degree is free from pleural adhesions and these adhesions are many times the reason that pneumothorax fails. Numerous illustrations of how they operate to hold cavity walls from collapse are to be found in any series of plates taken after this procedure. It is sometimes possible after repeated small injections of air to tear away small bands of adhesions or so to stretch them so that almost complete collapse follows. Jacobaeus of Sweden has introduced the use of an instrument akin to a large cystoscope and has met the difficulty of adhesions by opening the pleural cavity after such collapse as was

possible by artificial pneumothorax and cutting the restraining bands. How relatively easy this might be is illustrated by several of our cases, and while we have not as yet attempted it, we are convinced that in cases with few but effective adhesions successfully holding open a cavity, a trial of it is necessary as a step clearly indicated before thoracoplasty. That one is confronted with the danger of exciting a pyopneumothorax must be considered, for a cavity near the surface of the lung is often protected by a very thin wall and the release of a number of adhesions under pressure might easily put sufficient strain on the remainder to cause some giving way of the wall and the discharge of the cavity content into the pleural cavity.

That thoracoplasty is to be regarded as an ideal rest to a part that has reached a point where nothing else can affect the process, follows as a logical conclusion, and as the work has progressed in our hands, it has become more and more plain that when a lung is advanced in disease and pneumothorax is tried, thoracoplasty should follow if for any reason pneumothorax does not put the lung at rest.

II. SURGICAL ASPECTS (LEO ELOESSER)

It has been interesting to follow the changes in the points of surgical attack on pulmonary tuberculosis. Krause [1a] mentions the practice of artificial pneumothorax by the Hippocratic school. Sauerbruch [2] cites Willis as having cured tuberculous cavities in the seventeenth century by making a "fontanella" over the diseased side. Whether he opened the cavity or made an artificial pneumothorax is not apparent. In 1696, Baglivi wrote of introducing medicaments into "incurable internal ulcers of the lung" through an intercostal incision. In the eighteenth century a number of surgeons opened cavities with a lancet or trocar. These, I suppose, were cold abscesses from perforated cavities. In the eighties a number of authors resected portions of tuberculous lungs, and MacEwen [3] had a few brilliant results after the extirpation of entire purulent lobes.

With the exception of the ancient inductors of pneumothorax, and Willis with his "fontanella" over the diseased side, these surgeons were evidently following blind leads. Tuberculosis never makes an isolated tuberculoma of the lung: its lesions are diffuse. It is useless to remove one tuberculous patch and to leave many others behind.

Modern efforts to alter mechanically the course of pulmonary tuberculosis are based on observations of the effect of accidental complications (spontaneous pneumothorax and a pleuritic exudate) on the

---

1a. Krause: Tr. Am. Climatol. & Clin. Assn., Thirty-Ninth Annual Meeting, Washington, D. C., 1922; Am. Rev. Tuberc. **6**:154 (April) 1922.

2. Sauerbruch: Chirurgie der Brustorgane, Ed. 2 **1**:591.

3. MacEwen: Cavendish Lecture, West Lond. M. J. **11**:163 (July) 1906.

primary disease. A number of clinicians [4] had noted that neither pneumothorax nor an exudate were always untoward accidents, but that both sometimes arrested the underlying pulmonary disease. Spaeth,[5] in 1850, explained their benefits as being due to relaxation and immobilization of the lung. It was also a common observation that a chronic fibrous phthisis, with shrinkage of the lung, contraction and rigidity of one side of the chest, and displacement of the viscera toward the affected side, took a benign and slow course. To translate these two beneficial accidents, external compression of the lung, on the one hand, and internal shrinkage on the other, into practice was but a step. And yet, even after that sound basis had been established, the evolution of the present surgical procedures was by no means straightforward.

Forlanini,[6] in 1882, was the first to advocate imitation of a spontaneous pneumothorax by an artificial one. He struggled with his idea for six years before he was able to put it into practice. J. B. Murphy [7] followed him independently in 1898. Both Forlanini's and Murphy's ideas, however, lay fallow until the beginning of the present century when Brauer [8] took them up again and helped them to a secure footing.

It is to Brauer's rare combination of clinical insight and surgical imagination that we owe the first suggestion for our modern collapse operations. Convinced of the efficacy of collapse of the lung by pneumothorax in unilateral tuberculosis, he sought to extend the benefits of collapse to patients in whom pleural adhesions made pneumothorax impossible. In 1906, he suggested to his surgical colleague Friedrich that he might produce the effect of a pneumothorax by collapsing the whole chest wall. Friedrich [9] carried out the operation by resecting a long piece of all the ribs on one side, i. e., removing the bony frame of almost one half of the chest.

These two procedures, pneumothorax and thoracoplasty, underly our present efforts at a mechanical cure of pulmonary tuberculosis. Both procedures are calculated to compress the affected lung, to immobilize it, to rob it of its respiratory function, and to cause it to shrink. Forlanini's methods for pneumothorax have scarcely been changed in the forty years since their invention. The original Brauer-Friedrich extrapleural thoracoplasty, however, has been abandoned for less severe operations.

---

4. Emerson: Pneumothorax, Johns Hopkins Hosp. Rep. **11**:1, 1903. Cites Stokes: Diseases of the Chest, 1837. Houghton: Dublin J. M. Sc. **1**:313, 1832. Williams, C. J. B.: Diseases of Organs of Respiration, Philadelphia, 1871.

5. Quoted by Sauerbruch: Chirurgie der Brustorgane, Ed. 2, **1**, p. 594.

6. Forlanini: Gaz. d. osp. **3**:537, 585, 601, 609, 617, 625, 641, 657, 665, 689, 705 (Aug., Sept., Oct., Nov.) 1882; Therap. d. Gegenw. **49**:485, 531 (Nov., Dec.) 1908.

7. Murphy: J. A. M. A. **31**:151 (July 17) 1898.

8. Brauer: Beitr. z. klin. d. Tuberk. **12**:49, 1909.

9. Friedrich: Arch. f. klin. Chir. **87**:2, 1908.

Brauer was not the first to have suggested the extrapleural compression of tuberculous cavities. Others—Tuffier,[10] and especially Quincke [11] (we seem to owe most of our advances in lung surgery to the internists)—had tried it before him. It is to Brauer's credit, however, to have recognized the futility of trying to cure pulmonary tuberculosis by applying pressure over a small circumscribed area of lung. He was the first to see the necessity for collapsing the whole diseased lung and to build an operation on this principle.

Quincke (1888) and Spengler [12] had suggested the collapse of tuberculous cavities by resection of part of the upper ribs. Isolated attempts at this local collapse were made from time to time with more or less success until better insight into the effects of pneumothorax made it evident that a total collapse of the lung was necessary for a cure, and that partial operations would meet with but partial success.

The development of our present day procedures for the surgical treatment of pulmonary tuberculosis sheds much light on the rationale of some of them and the futility of others.

The main difficulty and the great interest of this treatment center in the niceties of indication for operation and the choice of procedure. The general indications for surgical collapse are easily formulated. A surgical collapse is indicated for those patients who should have a pneumothorax, but in whom a pneumothorax is impossible. That is an easy formula: it rolls the burden of the choice of patients onto the shoulders of the internist. If he wants to make a pneumothorax and finds he cannot, the surgeon takes his place. However, if the surgeon is to take the responsibility, he should make his own observations, together with the internist, and participate in the internist's conclusions. The surgeon will not regret keeping his patients under observation for several weeks before he makes up his mind. He should, by all means, have the advantage of frequent consultation with his internist during this time.

The indications for pneumothorax and for thoracoplasty are, then, largely the same, namely, a mainly unilateral process with a bad outlook for cure by ordinary conservative means. Physical signs and the roentgen ray, extensive unilateral cavities, repeated hemorrhages, multiple caseous pneumonic foci may often make it apparent from the beginning that the outlook is bad; again, it may take months to recognize that the patient is steadily getting worse in spite of good conservative treatment. It is not necessary that the tuberculosis be entirely unilateral, that one lung be perfect. Probably it never is. It is merely

10. Tuffier and Martin: Trait. chir. de la tuberc. pulm., Paris, Masson et Cie., 1910.
11. Garre and Quincke: Lungenchirurgie, Ed. 2, Jena, 1912.
12. Spengler: Verhandl. d. Naturforscherversammlung Bremen, 1890.

necessary that the better lung be competent to carry on the work of both, and that it contain no foci that are likely to light up under the additional burden, i. e., no large cavities or evidently active areas. Hemorrhages, large amounts of sputum, fever, do not contraindicate operation, but rather strengthen the indications.

A release of the lung by pneumothorax will be most strikingly effective in chronic fibrous phthisis with cavitation. Patients whose lungs have shrunk and drawn with them the mediastinal viscera, who are dyspneic from a kinking or a constriction of the trachea, or who are cyanosed from a displacement of their hearts and great vessels, will be given the greatest relief. They suffer not so much from their tuberculosis, but from deformities of their viscera arising from the process of its cure. The very shrinkage that is going on in the lung shows the cure. Sometimes the tuberculosis may be quite healed and only the deformities provoke symptoms. More often the cure is incomplete. Would the bony thorax but yield and let the lung contract completely, it would heal. It is the rigid chest wall that holds the lung out and keeps the cavities distended. Often one can recognize these patients at first sight. They are the ones who have one side of the chest sunken in, rigid, with no respiratory movement; whose one shoulder has dropped, whose spine is bent toward the affected side.

However, not only this fibrous phthisis, but also the more florid, suppurative and ulcerative form, may be amenable to cure if the affected lung is compressed, kept quiet and allowed to shrink.

Unfavorable for compression are miliary tuberculous disseminations.

I have detailed the indications for pneumothorax because, as stated, the same indications hold good for thoracoplastic collapse.

The chronic fibrous phthisics, whose retracted chests give evidence of the natural tendency to a cure, whose course shows the mildness of the disease or the patient's great resistance, are ones likely to be helped by a pneumothorax. But it is in just these patients that a pneumothorax is most frequently impossible. Their chests are shrunken and their mediastina displaced because the lung has everywhere grown fast to them. The same adhesive pleurisy that makes the chest shrink precludes the possibility of getting air into it. Most of our subjects for operation, therefore, will be recruited from this group.

Others, especially those with large superficial cavities in the upper lobe, will come for thoracoplasty after having had a pneumothorax—an incomplete one. Frequently one succeeds in introducing air into the lower part of these patients' chests, one collapses the lower part of the lung; but the upper part, the one containing the cavity, remains tightly fixed to the chest by adhesions.

Others will have open tuberculous empyemas. They have their pneumothorax; their lungs have healed. It remains to close the chest.

The contraindications are partly covered by the contraindications to artificial pneumothorax: too much involvement of the better lung and miliary tuberculosis. To these I might add unwillingness or reluctance of the patient to accept operation. The results are not certain enough to force operation on an unwilling patient. Unwillingness, however, is infrequent enough. More often will one have to reject patients who are unsuitable, even though they plead for operation, than urge the suitable ones to be operated on.

Sauerbruch warns us of an idiosyncrasy to morphin or cocain. He tells of a woman whom he rejected on this score. Another surgeon, ignorant of her peculiarity, operated on her. She died on the table.

If at all possible, the operations should be done under local anesthesia alone; the addition of a general anesthetic increases the risk greatly. It is not the irritation of the lungs by the anesthetic that seems to me so objectionable. The chemical irritation of nitrous oxide gas is negligible. It is the insensibility of the patient to safeguarding reflexes, his inability to cough, to regulate the depth of his respiration according to his momentary needs and the waste of muscular energy in useless struggling that make general anesthesia dangerous. Inspirations or expirations, unregulated by a waking brain and carried out at improper moments, suck and blow tuberculous sputum and pus from one part of the lung to another. The lack of a cough reflex stops the patient from coughing material out again once it has been aspirated. His useless struggles with his limbs and body and with his respiratory muscles wear out his sorely needed store of energy. These are the risks that the surgeon adds whenever he finds it necessary to supplement local anesthetics with gas.

Finally, a most important contraindication—no attempt has been made at artificial pneumothorax. Artificial pneumothorax is the procedure of choice for collapse of the lung. It is easier to do than thoracoplasty, less risky and accomplishes the same result, if it can be carried out. It has, moreover, an advantage over thoracoplasty in not being permanent. One can stop treatment after the tuberculosis is healed, and if the pneumothorax has not had to be maintained too long the air will gradually be absorbed from the chest and the lung will unfold. But a thoracoplasty collapses the chest for good and all.

At times, however, the permanence of a thoracoplastic collapse may be no disadvantage. At the last meeting of the California State Tuberculosis Association, I learned from members of large experience that insufflations of gas, once they have been begun, must be continued over very long periods. This, I think, is worth considering in weighing the desirability of a permanent collapse. Were I a tuberculous patient whom a pneumothorax had restored to well being but who relapsed as soon as the air in my chest was absorbed; were I a patient wedded—or

chained, if you like—to a doctor's doorstep by the need of a series of refillings to which I could see no end, it seems to me that I should be delighted at the prospect of an operation which would release me from medical surveillance. When clinical experience, therefore, shows the probable necessity for a very long continuance of pneumothorax; when after a year or more even the very gradual expansion of a previously active tuberculous lung is followed by renewed activity, it seems to me worth while to consider supplementing or supplanting the pneumothorax by a thoracoplasty. And all the more because previous gas compression of a lung makes surgical compression both technically easier and less dangerous.

One never can tell, in spite of roentgenoscopy and all manner of diagnostic attempts to show pleural adhesions, whether the lung is bound down tight or not, and whether a pneumothorax is possible or not, unless one tries it. Thoracoplasty, therefore, should never be done before pneumothorax has been tried, and has failed or proved insufficient.

The surgeon will usually place more reliance on the internist's opinion and diagnostic findings than on his own. And unless he is an uncommonly good diagnostician, he will be afraid to proceed alone without the help of a competent internist. But final judgment for or against operation; what, and how much to do, rests with the surgeon.

To know his own mind he should have the patient under observation for a reasonable time, at least a week or two, before operating. The surgeon's viewpoint is different from that of the medical man. No matter how accurate clinical observation has been, the surgeon should see and judge for himself. He will learn a great deal about tuberculosis, things which he never knew, or had forgotten. It is surprising how often simple observations, which are far more important surgically than the judgment of râles or resonance, are overlooked in patients with detailed charts and records. The shape and movements of the thorax especially: which part is retracted, which part moves and which stands still, inspiratory retraction in the intercostal spaces, the position of the trachea in the sternal notch, are often overlooked. The respiratory capacity of the lungs and the daily quantity of sputum should be recorded. All these factors are of considerable importance.

It is often as difficult to decide on the type of operation as it is to know whether to operate at all. There are several methods for total collapse, and several for partial collapse. Sauerbruch and others of experience seem agreed that partial collapse is to be used only in conjunction with the methods for total collapse, i. e., together with artificial pneumothorax, or after a total thoracoplasty has failed sufficiently to compress circumscribed areas of lung. Partial collapse of the upper lung alone, as a primary procedure, is dangerous and rarely leads to a cure. Disastrous aspiration of tuberculous pus into the lower parts

of the lung during operation on cavities in the apex has shown that the lower parts should always be collapsed first. Flooding the lower part of the lung with pus is the chief cause of postoperative mortality. This caution applies to nontuberculous abscesses of the upper lung as well.

There are two procedures for total collapse of the chest. The first, the Brauer-Friedrich operation, consists in the removal of large sections of the second to the tenth ribs. Under general anesthesia, a long fishhook shaped incision is made between the scapula and the spine and carried forward toward the nipple. The scapula and soft parts are pushed up from the chest and long pieces of rib are removed rapidly. This divests half of the chest of its bony support. It is evident that the procedure entails considerable shock and has alarming consequences. The patients breathe almost as though they had a wide open pneumothorax. The unresistant chest wall flaps back and forth with each respiration. For the first few days the dyspnea is alarming. It is necessary to steady the chest by various devices: turning the patients on the operated side, strapping the chest with adhesive, firm bandaging, etc. Unless one gives the chest some hold, the patients merely gasp ineffectually for breath. The bellows which move the lungs are too flabby to pump air in and out. If the patients survive the first few days, they gradually accustom themselves to the new type of breathing. Many of them, however, succumb to the intense air hunger. The operation carries with it too high a mortality to be of wide use. It may, however, still be indicated in patients in whom a thick or resistant pleura, or a thick and unyielding mediastinum, separates the chest into two distinct compartments; so that the one will be stiff enough to carry on the work of respiration even though the other be totally out of commission. Its sphere will probably be limited to the after-treatment and collapse of open tuberculous empyemas. In these cases it may be carried out after the manner of a Schede thoracoplasty with considerable prospect of success.

Sauerbruch's procedure has many advantages over Friedrich's and has superseded it. Sauerbruch collapses the chest by resecting smaller portions of all the ribs through a paravertebral incision. His resection is carried out close to the spine, at the costal angle. His procedure is much less mutilating; it diminishes the thoracic volume very nearly, if not quite, as much as Friedrich's operation, and it has the great advantage of producing collapse without robbing the chest of its rigidity —of its bony support. It is necessary, in order to get a good collapse, that the ribs be resected close to the spine. When this is done, the chest is narrowed not only in the transverse, but also in the sagittal diameter. The ribs not only cave in, but their sternal ends drop down nearer the backbone. The vertical diameter of the chest is lengthened. The chest

becomes narrower both fore and aft and sidewise, but it also becomes longer. The ribs run almost vertically instead of horizontally, the intercostal spaces are much narrowed, especially toward the back. The reasons are clear when one thinks of the way in which the ribs are suspended. Their fixation points lie at the two costovertebral joints: the joint between the head of the rib and the vertebral body, and the joint between the tubercle of the rib and the transverse tuberosity. These two joints determine the position of the ribs and hold them firmly out from the spine. The sternal end of the rib is flexible. One can cut the ribs off from one side of the sternum entirely and they will still maintain their position in a transverse plane fairly stiffly. If, however, one cuts the connection between the ribs and the spine on one

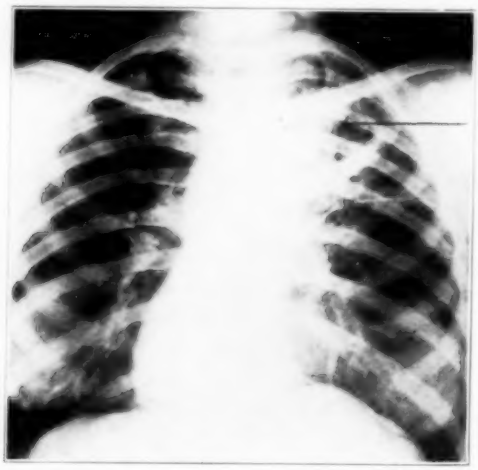


Fig. 1.—Before pneumothorax, showing large cavity of right upper lobe.

side, that side of the chest becomes freely movable and drops down by its own weight, the weight of the viscera suspended from it, and the pull of the abdominal muscles. This drop and the vertical narrowing may be seen by comparing Figures 9 and 10, and Figures 17, 18 and 20.

The amount of collapse produced by the resection of only a few centimeters of the posterior part of the ribs is astonishing. Compression occurs, as stated, in two directions—transverse and sagittal. The diminution in the sagittal diameter becomes apparent immediately the lower two or three ribs have been cut across. As we continue our resection upward, the collapse increases before our eyes. The chest wall falls in toward the midline more and more as we proceed. The

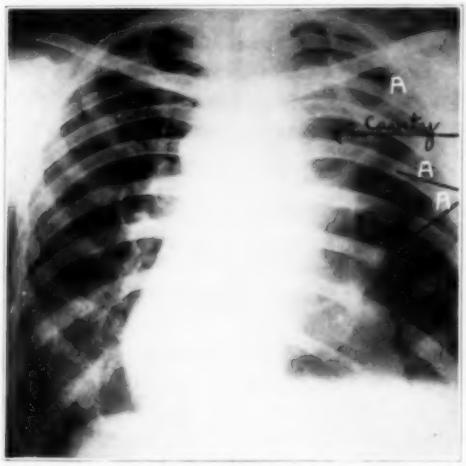


Fig. 2.—After pneumothorax, showing cavity still uncollapsed, its walls held distended by adhesions. A-Adhesions.

Fig. 3.—Before pneumothorax, showing large cavity with fluid level.

diminution in the other direction, the transverse decrease, which is caused largely by the dropping down of the ribs, does not become apparent until the two uppermost ribs are cut, especially the top one, which is the main support from which the chest is suspended. The collapse is striking as soon as the first rib is severed. The whole chest wall drops down suddenly, like a tent whose pole has been taken from under it. This drop does not occur until the first rib is cut through, but after that the whole chest drops downward several inches, one rib following the other like a series of card-houses. The downward shift usually covers from one and one-half to two intercostal spaces so that the sternal end of the first rib lies opposite the vertebral stump of the third rib, or even lower.

Sauerbruch's operation is by far the better procedure for total collapse. It is much less shocking than the Brauer-Friedrich's thoracoplasty. It can be done under local anesthesia, a very great advantage. It can be done in several stages. We can stop our resections at any time if the patient becomes dyspneic. There are no alarming after-effects. It does not upset the whole respiratory mechanism by robbing half of the chest of its bony framework.

It is really easier than it might seem. In the beginning I planned to do the operation in several stages. But I found it necessary to stop short only once in the five patients. In three cases I was able to resect pieces of all of the ribs, from the first to the twelfth, at one sitting; in one case, the first of the series, I left the first rib, ignoring its importance, and had to remove it at a second sitting.

The patients are not visibly deformed. No one would notice the deformity when they are clothed.

Following the ideas of Webb, the patients are laid on the operated side after operation so that the weight of the body may further the collapse and keep the affected side quiet. They are given morphin or codein, and are turned twice a day so that they may cough out the accumulated secretions. If possible, the posterior edge of the scapula is laid in front of the vertebral stumps of the ribs so that it may push the chest still further inward. The patients are kept in bed about eight weeks.

The chest is collapsed, as stated, both in the transverse and the sagittal diameter. There are no respiratory movements, except in the upper anterior part of the chest. The sternal ends of the first two or three ribs move. They are carried along by the sternum, I think, with the respiratory movements of the unoperated side. Below the scapula, where the resected ribs are most movable, the chest wall often moves paradoxically with respiration: that is, it is retracted on inspiration and moves out from its inspiratory position when the patient breathes out.

The collapsed side is dull to percussion, but on auscultation all sorts of sounds are heard: loud bronchial breathing and various squeaks, rubs and râles. All observers are agreed that it is very difficult to judge of the meaning of these physical signs. The bronchial breathing probably comes from the air in the bronchi causing a whistle to be transmitted loudly through the shrunken and solid lung. The squeaks and râles are partly pleuritic and partly produced in the atelectatic alveoli.

The mediastinum and heart, which before operation are often pulled far over toward the affected side, frequently return to the midline after operation.

The respiratory rate has not increased after operation.

We have had no untoward results. It is too early to speak of cures, but all of our five patients have improved. None has had elevation of temperature. The sputum has diminished in all of them. One patient has no sputum at all; two patients expectorate about 4 c.c. a day. Two still bring up from 15 to 30 c.c. a day. Two of those with sputum show no tubercle bacilli on repeated examinations. All of them have gained or held their weight, look well and are free from pain; their disease has been arrested or has diminished in extent.

We have been fortunate enough to avoid an aggravation on the better, unoperated side. Indeed, in some of the cases not only the operated but the unoperated side has been improved. The improvement was noticed clinically, and Dr. Bryan also called attention to the marked differences in the roentgenograms. We have been fortunate, but others have noted the same thing. They have tried to explain the improvement in various ways: they have said that the lung was like the kidney in that the less affected one improved as its neighbor improved; that the appetite and general resistance were benefited by the abolition of cavities and expectoration.

One can go farther than this. Stivelmann, Hennell and Golembe,[13] and Barlow and Kramer[14] seem agreed that there may be anatomic grounds for the bilateral improvement. It seems to me that we are far too prone to look on the two halves of the chest as two separate and independent compartments. They are not. Whatever changes the pressure on one side will change the pressure on the other. The mediastinum is not a sheet iron wall; it is an exceedingly thin and yielding membrane. One can see it change its position after operative collapse of the chest. The measurements of Simon[15] show that pneumothorax lowers the tension not only in the collapsed but in the contralateral half of the chest, and that the decrease is about equal on

13. Stivelmann, Hennell and Golembe: Am. Rev. Tuberc. **6**:95, 1922.
14. Barlow and Kramer: Am. Rev. Tuberc. **6**:75, 1922.
15. Simon: Tr. Nat. Tuberc. Assn., Seventeenth Meeting, 1921, p. 402.

both sides. Collapse, therefore, compresses not only the collapsed side but the other side as well; so that not only the affected lung, but also the unaffected lung, should reap some of its benefits. Releasing the shrunken side from the tug of its pleura should help the emphysema and distortion of the less affected lung, and put it in a better position to use its powers of shrinkage where they are needed, i. e., over its tuberculous areas.

In discussing the various operative procedures we stated that the total collapse of Sauerbruch was the operation of choice; that partial collapse was dangerous; and that we had been able in every case but one to resect pieces from all twelve ribs in one sitting. This is true; but a single resection is sometimes insufficient. Some cavities are not obliterated by it. The fault lies not with the operation, but with the cavity. One can always decrease the volume of the thorax sufficiently to relieve the lung of all tension. Some cavities, however, are so stiff-walled that they will not collapse even when there is no tension on them at all. These stiff-walled cavities have to be pushed in if they are to be obliterated. Their walls will never fall in by themselves. Here partial resection and partial compression enter the field.

There are various methods: the columnar resection of Wilms,[16] who removes a piece of rib both anteriorly and posteriorly; intrapleural apicolysis, where the hand is introduced into the pleural cavity and adhesions broken down; extrapleural apicolysis, where the pleura of the upper thorax is loosened from the chest wall together with the underlying adherent lung; and the methods of Tuffier [10] and of Baer,[17] who compress the lung with a free graft of fat or with soft paraffin, and of Archibald [18] who places a tampon of muscle over the cavity. Partial collapse is particularly indicated when an artificial pneumothorax succeeds in compressing the lower part of the lung, but where the apex and its cavities are held stretched out to the chest wall by firm pleural deposits. When a partial pneumothorax is possible, its combination with partial thoracoplasty is an ideal procedure. A partial pneumothorax compresses the lower part of the lung; after this a thoracoplasty may compress the upper part with impunity. There is no danger of aspirating material from an upper cavity into the lower lung after the lower lung has been compressed by a pneumothorax.

I have had to supplement Sauerbruch's thoracoplasty with an operation on the apex twice. The first patient had an enormous cavity with sputum up to almost a pint a day. Beneath the cavity she had a pyopneumothorax which Drs. Whitney and Richter, who were kind enough to send her to me, had aspirated and washed out at intervals.

16. Wilms: München. med. Wchnschr. **60**:449, 1913; **61**:865, 1914.
17. Baer, G.: Ztschr. f. Tuberk. **23**:209, 1914.
18. Archibald: Tr. Am. Surg. Assn., 1922.

A Sauerbruch resection obliterated the pyopneumothorax and lessened the diameter of the cavity by about one half, but did not obliterate it. The sputum dropped at first to about 50 c.c. a day, then rose again to about 100 c.c. Two months after the first operation, I resected an inch of the clavicle and considerable pieces of the upper four ribs through an anterior incision. The sputum diminished to about 30 c.c.; the cavity is much smaller but it is not yet collapsed. Of late her sputum has again increased and a cold abscess has broken through the chest from her old empyema. A third operation will probably be necessary.

A second patient operated on in two stages by the Sauerbruch procedure also retained a considerable cavity at her apex. At a third operation I removed an inch of clavicle and practically all that remained of the first and second ribs, as well as three and a quarter inches of the third rib.

REPORT OF CASES

CASE 1.—Mrs. M. M., a housewife, aged 29, was referred by Dr. Philip King Brown to the San Francisco Hospital, March 1, 1921. She was discharged, Dec. 22, 1921. She has two children.

History.—She had measles in March, 1920. Following this she complained of increasing weakness, cough and fever. For the past year she has had afternoon temperature of 99 and 100 F. She expectorates 25 or 30 c.c. of sputum a day. At times it is blood streaked. Various attempts at pneumothorax have failed.

Examination.—March 29, 1921, Dr. Clark examined her and found her to be undernourished. The thorax was long, flat and broad. The left shoulder was lower than the right, and the respiratory movement of the chest was restricted on both sides, decidedly more on the left side than on the right. The whole left side was dull; it was flat down to the seventh dorsal vertebra and less dull below. It was dull in the left axilla. On the right side there was dulness above the clavicle and posteriorly down to the second dorsal vertebra. The right side of the diaphragm moved fairly well; the left side moved very little. There were râles in the upper left chest on coughing, with amphoric breathing. There were also râles below the left clavicle. There were râles over the right clavicle and over the right fifth rib. The heart was displaced to the left.

TABLE 1.—SUMMARY OF DATA IN CASE 1

	Temperature, F.	Pulse	Respiration	Sputum, C.c.	Weight, Pounds
Before operation	98.6-100-102	80-110	20-35	25-30	112
Postoperative abscess, April 14-20	98-101-102	80-140	30-45	?	?
April 26 to July 1	98-99.5	90-120	20-40	6	104
July 1 to August 1	98-99	90-120	30	0-5	?
August 1 to September 1	98-99	80-100	25-30	10	104-114
Second operation, September 19-25	98.6-102	90-120	25-40	10	?
September 25 to December 22	97.8-98.6	85-100	22-25	5?>0	?
Discharged, April 13, 1922	98-98.8	.....	.....	0	134

Sputum: March 2, 1921, Tb. +; Aug. 26, 1921, Tb. +; Oct. 28, 1921, negative.
Urine negative.
Blood: Hemoglobin, 80 per cent.; red blood cells, 5,000,000; white blood cells, 8,850 to 11,850; polymorphonuclears, 62 to 72 per cent.; small lymphocytes, 38 to 25 per cent.; Wassermann negative.

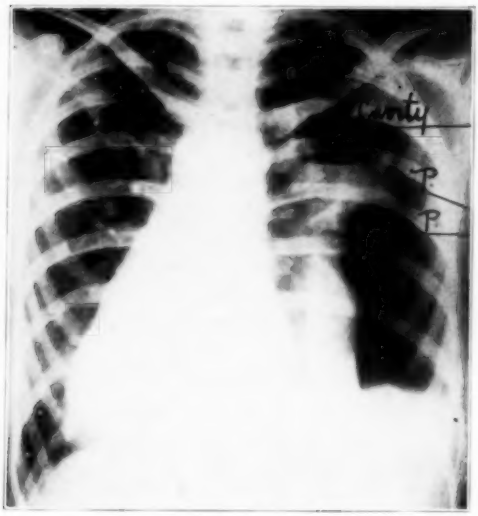


Fig. 4.—After pneumothorax, showing cavity held distended by adhesions, still uncollapsed. P-Pneumothorax.

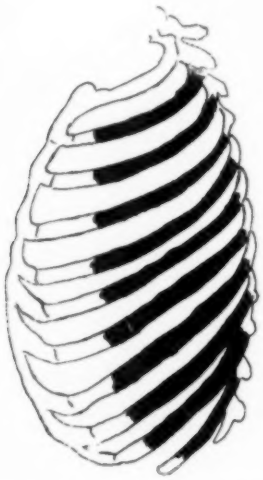


Fig. 5. Amount of ribs resected in Friedrich operation.

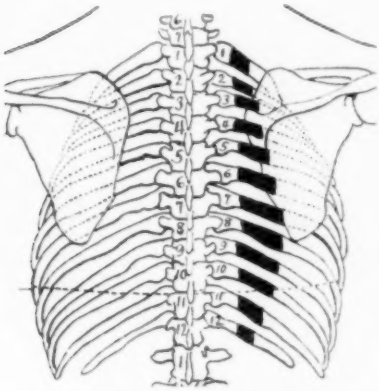


Fig. 6.—Amount of ribs resected in Sauerbruch operation.

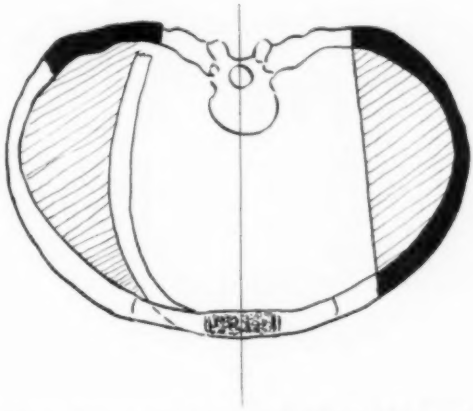


Fig. 7.—Diagram of cross-section of chest. Friedrich's resection on the right side, Sauerbruch's on the left; amount of rib removed shaded black. Although the amount of rib resected in the Sauerbruch operation is much less, the collapse is almost, if not quite, as great as after the Friedrich operation.

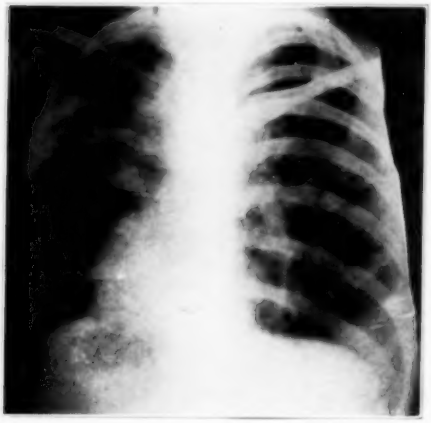


Fig. 8 (Case 1).—Before operation. Large cavity left apex. Infiltrate below. Considerable mottling right side.

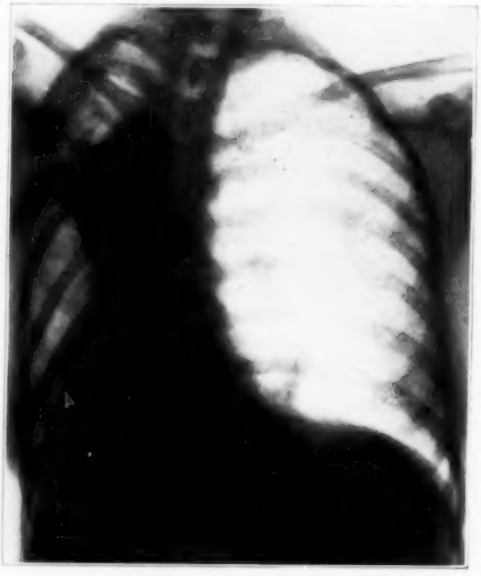


Fig. 9 (Case 1).—Five months after first operation. First rib not resected. Collapse incomplete. No sinking downward of left chest. Cavity of left apex is smaller, but is not yet collapsed. Scoliosis to left and compensatory dilatation of right lung.

Roentgen-ray examination, March 21, 1921, disclosed a cavity in the upper left side of the chest and an infiltration on the left side.

Diagnosis.—Cavities in left side. Thick pleura left base. Thick pleura right apex. Active tuberculosis upper and middle lobe, right.

Treatment.—First Operation: April 14, 1921, under satisfactory local anesthesia from the second to the eleventh ribs were resected according to Sauerbruch's method. The resected pieces measured as follows: Second rib, 3.75 cm.; third rib, 3.75 cm.; fourth rib, 3.25 cm.; fifth rib, 3.25 cm.; sixth rib, 3.5 cm.; seventh rib, 2.75 cm.; eighth rib, 2.6 cm.; ninth rib, 2.25 cm.; tenth rib, 1.75 cm.; eleventh rib, 0.5 cm. The pleura was opened and cobweb adhesions were seen. No pneumothorax resulted. The lung was grasped with a hemostat and pulled about. This was done to find the cause of the pain in adhesive pleurisy. The patient, however, felt no pain during this maneuver. After cutting the second rib, the chest as a whole dropped back toward the spine. The medial edge of the scapula was placed under the stumps of the ribs.

Fig. 10 (Case 1).—Four and one-half months after second operation. Cured. First rib resected; additional pieces removed from second and third ribs. Left chest has sunk down so that peripheral stump of first rib lies opposite central stump of third rib. Peripheral stumps of first and second ribs united by bony callus. Cavity completely collapsed. Note the difference between Figures 9 and 10 (before and after resection of the first rib). After resecting the first rib, the upper chest is much narrower and the cavity completely collapsed.

Course.—April 15, the heart had returned to its normal position: the apex was in the left nipple line and the right border was at the right sternal edge.

April 25, some pus containing staphylococci appeared in the wound. It contained no tubercle bacilli (guinea-pig inoculation).

August 23: Up to ten days ago the patient had no sputum. She now has 1 c.c. per day. After "catching a cold" she had more sputum and cough.

September 16: Roentgen-Ray Examination: Medial part of left diaphragm moves about one-half inch. There is a small area of mottling at the base of the right upper lobe with interlobular thickening.

September 17: The cavity at the left apex still remains.

Second Operation: September 29, a second operation was undertaken. Under satisfactory anesthesia the first, second and third ribs were resected. The second and third were joined by callus. The resected pieces measured: First rib, 3 cm.; second rib, 5 cm.; third rib, 2.5 cm. After resection the apex collapsed. Part of it was hard, but the top of the apex was soft and blew in and out on respiration as though it contained a thin walled cavity.

September 22: The patient was discharged to convalescence at Arequipa. She felt well, was gaining weight, and had no sputum.

April 13, 1922, she was finally discharged. She has gained 24 pounds since her first admission. Her color is good, she looks robust and sturdy. She is now doing housework, has no cough, no sputum, and may be considered cured.

Case 2.—Miss E. W., a nurse, aged 28, was referred by Dr. Philip King Brown to the San Francisco Hospital where she stayed from March 29 to Aug. 6, 1921.

History.—One brother died of tuberculosis. At the age of 3 she had pneumonia and at 14 she had "grippe." She was in bed and was kept out of school for six months, and did not feel well during this time. Six years ago she had an hemoptysis of a few ounces. This recurred several times, the last time on Christmas day, 1920. For the last six years she has had afternoon rise in temperature at times; in February, 1921, her temperature was 100 and 101 F. Three years ago she had a pleurisy which lasted off and on for two years. For the last one and one-half years she has had to sit up in order to breathe. She had four attacks of dyspnea, each lasting three weeks, and at this time she had fluid in her chest. She was at Arequipa twenty-two months with daily sputum measuring from 25 to 50 c.c. For the past eleven months she has been at the Marin County Infirmary and has had from 50 to 100 c.c. of sputum a day. Eleven attempts at pneumothorax were made at Arequipa but were only partially effective, as follows:

1919.	Feb.	8,	75 c.c. air		April	5,	no air
	Feb.	15,	75 c.c. air		April	8,	65 c.c. air
	Feb.	22,	190 c.c. air		Dec.	27,	100 c.c. air
	March	1,	225 c.c. air				
	March	8,	350 c.c. air	1920.	Jan.	3,	65 c.c. air
	March	15,	225 c.c. air		Jan.	10,	100 c.c. air
	March	22,	140 c.c. air		Jan.	24,	225 c.c. air
	March	29,	no air		Jan.	31,	50 c.c. air

While at Arequipa she gained 33 pounds in weight (137-170 pounds) but had frequent hemorrhages. Lying on her right side makes her cough and bring up more sputum. She sleeps on her back or on her left side.

Examination.—May 3, 1921, Dr. Clark examined her and found a well nourished girl with a sunken right chest. The whole right side was dull, except just under the clavicle. There was amphoric breathing at the right apex with bronchial breathing at the right base and right hilus. There were bubbling râles on the right side. The left side was hyperresonant and there were moist râles at the left base (communicated from right?). There were no râles over the left upper chest.

Dr. Eloesser's examination reads: Right side moves, but less than the left. The right clavicle does not rise as much as the left, and the right chest excursion is less than the left. The upper right quadrant, under the pectorals, rises well.

Diagnosis.—Chronic tuberculosis. Cavity of the right lung.

Treatment.—Before operation the sputum measured from 50 to 100 c.c. For the first four weeks after operation it averaged 20 c.c. a day, then 15 c.c.

Fig. 11.—Case 1. After operation. Collapse of left side. Gain in weight. Case 2. After operation. Collapse of right chest. Marked scoliosis. Case 3. After operation. Collapse of right chest. Marked scoliosis.

Fig. 12 (Case 2).—Before operation. Before pneumothorax. Pleurisy with effusion right base.

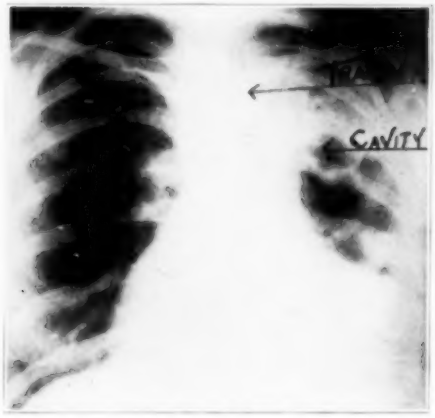


Fig. 13 (Case 2).—Before operation. Effusion absorbed. Large cavity middle right lobe. Trachea drawn to right.

Fig. 14 (Case 2).—Fifteen days after operation. Distal end of first rib in first interspace. Collapse incomplete. Considerable gap between ends of resected ribs. Trachea is drawn to right. No cavity seen.

Fig. 15 (Case 2).—Seven weeks after operation. Collapse much more marked, especially around middle and lower parts right chest. Distal end of first rib in first interspace. Heart has moved to the left. Trachea is drawn to right.

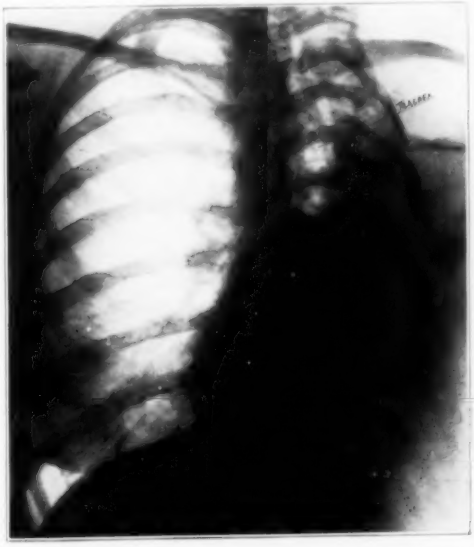


Fig. 16 (Case 2).—Ten months after operation. Peripheral end of first rib in first intercostal space. No further signs of cavity. Good collapse, especially of upper part of chest. Trachea is drawn to the right. The usual scoliosis is absent. This figure is a combination of two films: the one overexposed to show the collapsed side; the other with a normal exposure for the good side.

May 4, 1921, under satisfactory local anesthesia, the ribs were resected. The resected pieces measured: First rib, 1.5 cm.; second rib, 2.7 cm.; third rib, 4.5 cm.; fourth rib, 7.3 cm.; fifth rib, 6.75 cm.; sixth rib, 7.5 cm.; seventh rib, 8 cm.; eighth rib, 8 cm.; ninth rib, 8 cm.; tenth rib, 6.2 cm.; eleventh rib, 6.2 cm.; twelfth rib, 4.5 cm. The collapse of the chest began to be evident after removing the ninth rib. After removing the fourth, fifth and sixth ribs, those overlying the cavity, the patient began to expectorate large quantities of sputum. The collapse amounted to from 3 to 4 inches in the transverse diameter.

Course.—Miss W. made a smooth convalescence and was discharged from the San Francisco Hospital, Aug. 7, 1921, with an average of 15 c.c. of sputum a day and normal temperature.

She was readmitted to Arequipa for convalescence, August 15, and while there had sputum averaging from 10 to 12 c.c. a day. Between Dec. 5, 1921, and May 1, 1922, during which time she worked in the laboratory, she made twelve careful examinations of her own sputum and found no tubercle bacilli. She was up twelve hours a day.

May 21, 1922, she stated that since November, 1921, when she had a cold, she had been sleeping on the good side because she found it easier to breathe and because she coughed when she lay on her bad side. The right side showed a marked inspiratory retraction (paradoxical breathing) under the scapula. On coughing or making a forced expiration, the right side bulged out. There were amphoric breathing and moist râles in the right axilla.

TABLE 2.—Summary of Data in Case 2

	Temperature, F.	Pulse	Respiration	Sputum, C.c.	Weight, Pounds
Before	98-100	70-100	20-25	50-100	172
After	98-99	80-100	20-25	20>15	163

Urine negative.
Blood: Hemoglobin, 80 per cent; red blood cells, 4,100,000.
Sputum: May 1, positive; June 23, negative; August 2, 3, and 4, negative for Tb.

Except for the 10 c.c. of watery sputum, without tubercle bacilli, which she brings up every day, the patient is cured. She is up every day, feels well, and is going East next week.

Twelve additional examinations, made from Dec. 5, 1921, to May 1, 1922, were negative. Spirometer readings: notes lost.

Case 3.—Miss T. T., student, aged 25, was referred by Dr. Philip King Brown to the San Francisco Hospital. She stayed from July 8, to Oct. 12, 1921.

History.—Her illness began after an attack of influenza, in December, 1919. In June, 1920, she had two hemorrhages. She entered Arequipa in September, 1920, but in spite of careful conservative treatment lost ground steadily. She is nervous, her heart is irritable, and there is possibly an element of hyperthyroidism. On admission to Arequipa her temperature was 100.4 F.; it rapidly receded to 98 and 99 F., and rarely reached 100 F. Three attempts at pneumothorax were unsuccessful; at a fourth a pleural pocket was injected with 75 c.c. of air.

Examination.—She was admitted to the San Francisco Hospital, July 8, 1921. The right side of the chest was contracted and scarcely moved on respiration. There was a diffuse fibrous thickening of the right lung with at least three large cavities. There were diffuse bubbling râles all over the right side. There was an audible wheezing stridor. The right diaphragm was adherent to the lung and moved only very slightly. The sputum measured 40 c.c. Spirometric measurements were not made.

Treatment.—First Operation: July 19, 1921, thoracoplasty was done under satisfactory local anesthesia, the fourth to twelfth ribs being resected. They measured: Fourth rib, 14.7 cm.; fifth rib, 13.3 cm.; sixth rib, 12.5 cm.; seventh

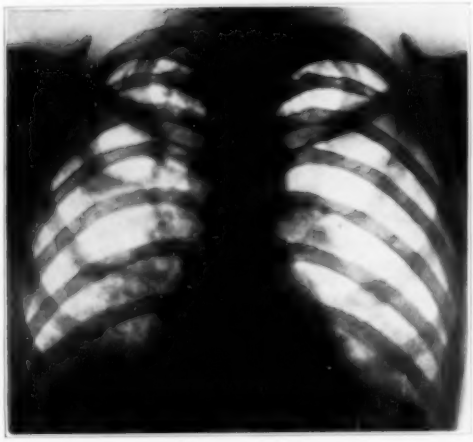


Fig. 17 (Case 3).—Roentgenogram made before operation. Three large cavities without fluid level in right upper and middle lobes. Increased peribronchial thickening in left lung. Right side is shrunken. Trachea is pulled to the right. No scoliosis.

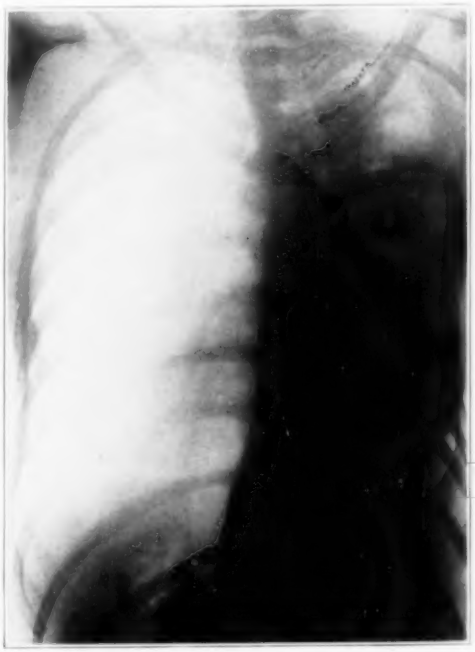


Fig. 18 (Case 3).—Three weeks after first operation. Three upper ribs remain. Upper chest uncollapsed. Trachea and heart still pulled to right. Slight dextroconvex dorsal scoliosis. This is seen to increase in the following figures.

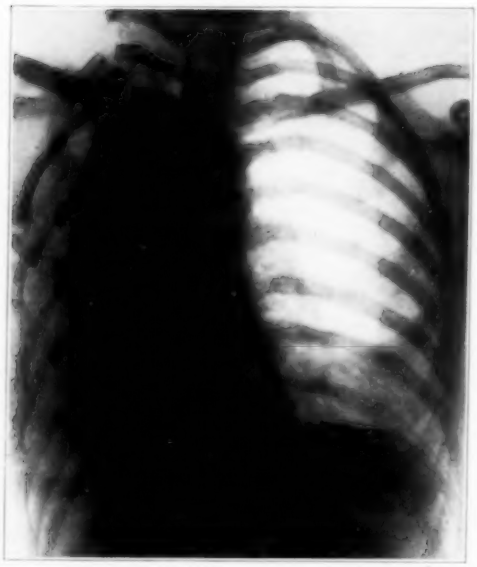


Fig. 19 (Case 3).—Six weeks after second operation. All ribs resected. Distal piece of first rib is opposite central stump of second.

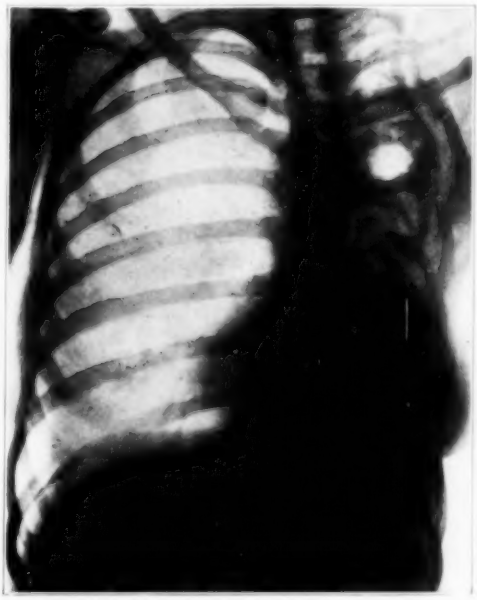


Fig. 20 (Case 3).—Five and one-half months after second operation. Extensive regeneration of resected ribs. Distal pieces of first and second ribs lie in second intercostal space and are welded to the central stump of the second rib by bony union. Bony union between the central stumps of the first and second ribs. Large cavities remain at the right apex and in the right middle lobe.

rib, 14.1 cm.; eighth rib, 11.7 cm.; ninth rib, 11.2 cm.; tenth rib, 11.5 cm.; eleventh rib, 8.8 cm.; twelfth rib, 5 cm. After the fourth rib had been reached the patient became dyspneic and it seemed safer to stop the operation.

Course.—The dyspnea continued to distress the patient for several days, but by the end of a week she was breathing easily. The sputum averaged 20 c.c. a day.

SECOND OPERATION: Aug. 31, 1921, the vital capacity measured 1,550 c.c. On this date the remaining three upper ribs were resected as follows: First rib, 2 cm.; second rib, 4 cm.; third rib, 6.9 cm. The patient stood the second operation well. Breathing was not labored. The following week she suffered considerably from dyspnea.

Fig. 21 (Case 3).—Six weeks after third operation. The clavicle has been resected and has united. Only the central ends of the first and second ribs are visible; their distal ends seem to have been removed completely. Extensive regeneration of resected ribs. The cavities are much smaller, but still plainly visible. Trachea seems to lie in midline. Marked dextrodosal sinitrolumbar convex scoliosis; this has developed in the eight months intervening since Figure 17 was made.

Course.—Oct. 7, 1921, the notes read: Left shoulder is lower than right. Sinistrolumbar convex, dextrodorsal convex scoliosis. Inspiratory retraction below right scapula. Heart beat visible and palpable on the right side, midaxillary line, fifth intercostal space. Lower part of chest does not move on inspiration. Upper part is lifted just a little. Dulness on right, except just over clavicle. Amphoric breathing and egophony here, and at the lower angle of the scapula. The rest of the right side is silent, except for a faint pleural rub. The left side shows only a few isolated râles and a pleural rub.

Oct. 12, 1921, the vital capacity measured 1,400 c.c. (a reduction of 150 c.c. from the reading before the second operation). The sputum averaged 17.5 c.c. a day and September 21 still contained tubercle bacilli.

She was discharged to Arequipa Oct. 13, 1921. During the first half of November she brought up from 15 to 20 c.c. of sputum per day. In the midde of November, after a coryza, the sputum increased to 25 and 40 c.c. The cavity was still visible under the fluoroscope.

THIRD OPERATION: Jan. 22, 1922, Dr. Brown sent her to the St. Francis Hospital for a third operation. This was performed Jan. 31, 1922, about one inch of the left clavicle and practically all that remained of the first, second and third ribs being resected under satisfactory local anesthesia. The resected bones measured as follows: Clavicle, 2.5 cm.; first rib, 3.7 cm.; second rib, 8 cm.; third rib, 9.4 cm.

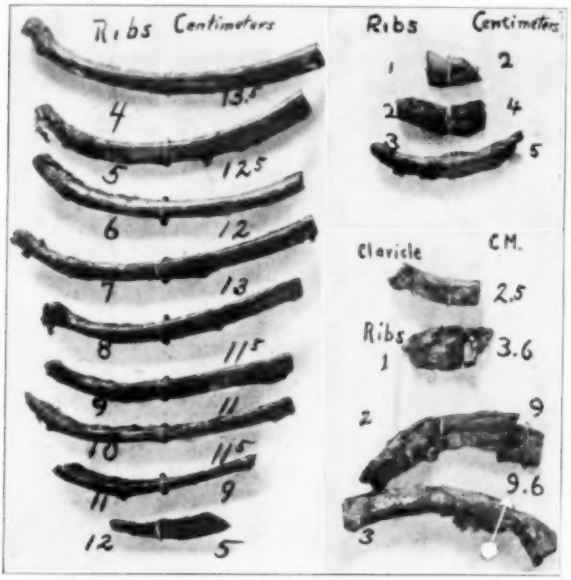


Fig. 22 (Case 3).—Ribs resected at first, second and third operations.

Course.—The patient suffered considerably from dyspnea for the first few weeks following operation, but made an otherwise uneventful recovery and was discharged to Arequipa, March 16, 1922. Her sputum measured from 3 to 25 c.c. per day, averaging about 15 c.c. Her temperature was normal.

TABLE 3.—SUMMARY OF DATA IN CASE 3

	Temperature, F.	Pulse	Respiration	Sputum, C.c.	Weight, Pounds
Before operation	98-99.5	80-100	20-30	40	128
July 18 to Aug. 30, 1921	98-99.5	100	20	20	124½
September 1 to Oct. 13, 1921	98-99.5	100± —	20-30	17.5	123<127

Sputum: Tb. positive Sept. 21, 1921, and June 20, 1922.

Examination, May 21, 1922, showed the right side standing still, except in the very upper part. There was an inspiratory retraction of the soft parts under the right scapula. There were no bubbling râles anywhere. There was a fine pleural rub at the left base. Her breathing was still wheezy. She was slowly gaining weight. The outcome seems doubtful.

CASE 4.—Miss K. O., a seamstress, aged 45, was referred by Drs. C. M. Richter and J. L. Whitney, and entered the San Francisco Hospital, July 30, 1921.

History.—Tuberculosis was discovered ten years ago. About 1915 she broke down completely: with increasing cough, fever and hemoptysis, and lost 10 pounds. She stayed at Arequipa fourteen months, and improved; then went to Los Gatos for seven months, at the end of which time she weighed 180 pounds; she then returned to Arequipa for five months. She came to San Francisco in February, 1919, and felt quite well. She had a moderate amount of sputum, some dyspnea, and occasional fever. By the end of 1919, she had lost 12 pounds and had fever, dyspnea and cough. From February to October, 1919, she had an artificial pneumothorax (fifteen injections) and felt well, but

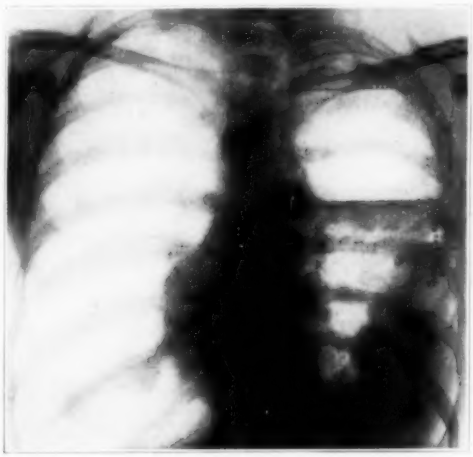


Fig. 23 (Case 4).—Before operation. Large cavity upper right with fluid level. Pyopneumothorax below with fluid level and small gas bubble. Heart and trachea drawn far to right.

the sputum remained the same. Nov. 8, 1920, she had pleurisy and fever. From January to April, 1921, pus was removed from the chest by Drs. Richter and Whitney and the chest was irrigated. Her average weight was 160 pounds; weight on entrance to the San Francisco Hospital was 135 pounds.

Examination.—The right chest is retracted and moves less than the left. There is a little expansion in an anteroposterior direction on the right side of the sternum, at the third and fourth ribs, but there is no anteroposterior expansion of the lower right chest. There is no transverse expansion on the right side. There is a cavity at the right apex and below it a pneumothorax with a succession splash. The mediastinum lies one inch to the right of the midline. The trachea enters the sternal notch one-half inch to the right. The respiration is wheezy. She expectorates mainly in the morning, on sitting up from a recumbent position. When sleeping on the left side she coughs, but attempts to make her cough by lying on her left side, or by hanging her head over the bed, are unsuccessful. Her heart is displaced to the right. Her

Fig. 24 (Case 4).—One month after operation. Distal stumps of first and second ribs in second intercostal space. Cavity persists, but is smaller; no fluid level. Dense shadow lower right chest. Trachea not distinct; probably to left of midline.

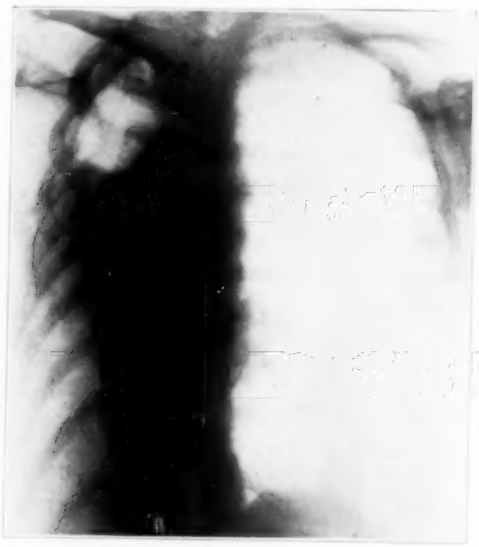


Fig. 25 (Case 4).—Nine weeks after operation. Cavity persists but is smaller. No fluid level. No evidence of fluid lower chest.

fingers are clubbed. Her sputum averages 140 c.c. per day. Aspiration in the seventh intercostal space, below the scapular angle, yields 30 c.c. of pus swarming with tubercle bacilli. On forced expiration no air comes from the needle, but a little bubbles in when the patient breathes quietly.

Diagnosis.—Chronic fibrous phthisis, with large cavity in upper right lung and pyopneumothorax below.

Aug. 11, 1921, some methylene blue was injected into the empyema cavity which was not coughed out in two days, showing that there was no bronchial fistula. August 17 the urine was stained green.

Treatment.—First Operation: Aug. 15, 1921, under satisfactory local anesthesia, the twelfth to the first ribs were resected. More was taken of the third, fourth and fifth ribs in order to compress the upper parts of the lung more.

The resected pieces measured: First rib, 2 cm.; second rib, 4 cm.; third rib, 6 cm.; fourth rib, 8 cm.; fifth rib, 8.7 cm.; sixth rib, 11.4 cm.; seventh rib, 7.8 cm.; eighth rib, 7.2 cm.; ninth rib, 7.5 cm.; tenth rib, 7.3 cm.; eleventh rib, 5.7 cm.; twelfth rib, 4 cm.

Course.—A week following operation the sputum measured from 20 to 25 c.c. A month later the sputum began to increase in amount, and Sept. 27,

TABLE 4.—Summary of Data in Case 4

	Temperature, F.	Pulse	Respiration	Sputum, C.c.	Weight, Pounds
Before first operation, Aug. 15, 1921....	97–99.5	80–105	20–28	140	135
August 16 to September 1.....	98.0	90	20	33	?
September 1 to October 1.....	97–98.6	80–90	25	120*	?
October 1 to November 12.....	97–98.6	75–90	25	110	125
Second operation, Nov. 12, 1921					
Nov. 12, 1921, to Jan. 1, 1922.....	97–98	85–95	25	40	128
January 1 to March 9.....	97–98.6	75–80	25	30	<133
Influenza.					
March 9 to March 20.....	98.6–101>98–99	85–100	20–25	125	133
March 20 to June 1.....	97–98.6	80–90	20–25	105	133

Blood: Hemoglobin, 75 per cent.; red blood cells, 5,500,000; white blood cells, 15,000; polymorphonuclears, 63 per cent.; small lymphocytes, 33 per cent.; large lymphocytes, 2 per cent.; mononuclears, 2 per cent.; basophils, 0.5 per cent.

* Increased from 30 to 200–400 toward last week of September.

1921, a distinct succussion splash was heard at the sixth rib. The patient had noticed this herself. Oct. 3, 1921, the chest expansion was, 75-77.2 cm.= 2.2 cm.

Roentgen-ray examination, Oct. 21, 1921, showed no movement of the diaphragm on Valsalva's experiment.[19] The right side of the chest was absolutely quiet. The cavity was not influenced by normal pressure on the chest.

Nov. 12, 1921, the signs of cavity and increased expectoration still persisted.

Second Operation.—Under satisfactory local anesthesia a curved incision was made over the middle of the right clavicle and carried downward to the fourth rib. The clavicle was cut through with a Z-shaped incision and the vessels and plexus were retracted. The fourth to the first ribs were resected from the mamillary to the axillary line. The site of the previous posterior resection had united so that the middle piece of the ribs could not be avulsed when they were cut through in front. The chest was well collapsed over the third and fourth ribs. The first two ribs, however, bulged out over the level of the flat chest like a dome. The clavicle was united with kangaroo tendon.

The resected clavicle measured 2.8 cm.; first rib, 2 cm.; second rib, 4.7 cm.; third rib, 5.5 cm.; fourth rib, 6 cm.

19. Forced expiration against the closed glottis.

Fig. 26 (Case 4).—Five weeks after second operation. Collapse much increased, especially of upper part of chest. A small narrow cavity still visible here. Marked improvement right lung. Trachea distinctly to right of midline. It is kinked to the right at the level of the top of the sternum.

Fig. 27 (Case 4).—Seven months after second operation. The right chest is much narrower and the ribs steeper than in the previous figure. The difference is especially noticeable in the lower part of the chest. The ribs run almost vertically. The peripheral ends of the first and second ribs lie in the second intercostal space. The trachea is in the midline. The clear space below the right clavicle is possibly, but not certainly, the remains of a cavity. The clavicle has united. The figure is a combination of two films: one overexposed for the collapsed side; one normally exposed for the good side.

Course.—Dec. 3, 1921, the patient sat up. The sputum was 30 c.c. per day. By December 7 the sputum had increased to 60 c.c.

Jan. 20, 1922, the roentgen-ray showed a satisfactory compression. The right diaphragm was obscured. The heart shadow was compressed against the right rib cage.

February 16, the roentgen-ray showed no respiratory expansion on the right side. The heart and mediastinum were displaced to the right. The left chest was normal in appearance and respiratory excursion (Dr. Chamberlain).

March 6, she had otitis media, and March 10, she had an attack of influenza.

March 24, the roentgen ray showed that the right side of the diaphragm moved slightly in the same direction as the left side. The mediastinum did not shift during expiration. The right chest was completely collapsed. There was no aeration of the right lung.

April 27, the vital capacity was 1,250 c.c.

May 12, the roentgen ray showed that the right diaphragm moved less than the left, but in the same direction. The right lung was well collapsed. The mediastinum moved to the right on coughing. There was a dense pleural scar in the lower right axilla.

Fig. 28 (Case 4).—July, 1922, eight months after second operation, showing retraction of right chest.

June 2, roentgen ray showed no collapsing spaces (cavities) in the right chest. There were old scars of the left hilus and lung. The right side of the diaphragm moved with the left as far as it could be seen.

June 24, she had a subcutaneous tuberculous abscess under the right scapula. The abscess was aspirated. There were no signs of empyema.

The sputum showed tubercle bacilli August 2, September 20, September 30 and Oct. 31, 1921.

The urine was negative. The Wassermann reaction was negative.

Additional Notes.—The patient is still in the hospital. The subcutaneous cold abscess which appeared June 24 doubtless came from a breaking through of the old empyema, although she has had no other empyemic signs. The succussion splash disappeared after the first collapsing operation and was last heard Sept. 28, 1921 (six weeks after the first operation). We thought that the continued sputum might be due to a perforation of the old empyema into the lung, but repeated examinations with this in view gave no positive evidence.

July 19, 1922, the cold abscess was incised under local anesthesia and about 100 c.c. of green pus with thick flakes of fibrin were removed. The incision was closed. Immediately after closing the incision the patient coughed and the abscess refilled; it doubtless communicates with the old empyema but not with the lung. It was irrigated with iodin; the patient neither coughed out the iodin nor tasted the fumes.

The cavity is smaller, but still persists. A third operation, probably an intrapleural direct compression of the cavity, may be undertaken. The patient is up, has no fever, and looks and feels much stronger than before operation. The outcome is doubtful.

CASE 5.—Miss B. N., student, aged 26, was referred by Dr. Philip King Brown to the San Francisco Hospital. She was in the hospital from Oct. 4, 1921, to March 16, 1922.

History.—The patient lived in the same house with a sister who died of tuberculosis twelve years ago. She has had a cough off and on for six years, which has been incessant for the last three years, with occasional blood-tinged sputum. She was in New Mexico for two years and ten months, and in July, 1920, entered Arequipa; in October, 1920, she was discharged unimproved.

During August and September, 1920, Dr. Mentzer made three attempts at pneumothorax. On the first attempt 100 c.c. of air went into the neck; on the second attempt, 250 c.c. of air went into the neck; and on the third attempt, all the air (250 c.c.) went into the neck.

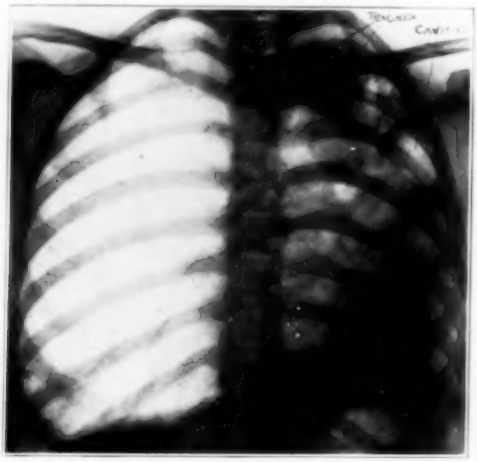


Fig. 29 (Case 5).—Before operation. Multiple cavities upper left. Trachea strongly retracted to left and curved at its entrance under the sternum. Calcified areas right.

Her tonsils were removed Oct. 2, 1921; before that she had occasional attacks of sore throat.

She was admitted to the San Francisco Hospital Oct. 4, 1921.

Examination.—The left side moved less than the right. There were fair movements above (second and third intercostal spaces), in an anteroposterior direction, but the transverse movement was small, one half that of the right side. The trachea entered the sternal notch one-half inch to the left. There was flatness from the middle of the left scapula downward, and there were creaks and râles all over the left side. The heart beat was palpable posteriorly below the angle of the scapula, at the bottom of a space that gave amphoric breathing and a clucking sound synchronous with the heart beat. There were crepitations at the right apex (possibly pleural) and a few fine râles after coughing. The blood pressure was 112/65. The roentgen ray showed dif-

Fig. 30 (Case 5).—Six weeks after operation. Cavities no longer visible. Trachea straight, but still slightly to left of midline. No scoliosis. Peripheral stump of first rib is in third interspace.

Fig. 31 (Case 5).—Four and three-quarter months after operation. March 21, 1922. Marked scoliosis toward the affected side. Trachea is in midline. Increasing dilatation is noted in right side of chest. Stump of first rib is in second interspace. Central stumps of first, second and third ribs and distal stump of first rib are united by bony callus. The left chest, especially at its top, is narrower than in the previous figure.

fuse fibrosis on the left side, and considerable infiltration and calcified masses at the right top. The chest expansion over the lower scapula was 79-77 cm. = 2 cm.; the vital capacity, 1,800 c.c.

Treatment.—November 1, resection of all the left ribs at the costal angle was done. The heart lay beating between the scapula and the posterior axillary line, being drawn far toward the back and the left side; the action was stormy. With the patient on her right side, the second rib lay higher than the first. The lateral part of the latissimus dorsi was inverted with two or three mattress stitches so that its cut edge was under the central stumps of the ribs. This was done to aid the dislocation of the posterior edge of the scapula forward under the posterior stumps of the ribs. The patient was returned to bed with a thin fleeting pulse and shallow respiration.

The resected ribs measure: First rib, 2.5 cm.; second, 4.3 cm.; third, 8.3 cm.; fourth, 8.2 cm.; fifth, 9.6 cm.; sixth, 10 cm.; seventh, 10.7 cm.; eighth, 8 cm.; ninth, 8 cm.; tenth, 7.5 cm.; eleventh, 7.8 cm.; twelfth, 3.5 cm. The following day she had a very rapid pulse, but was better toward evening.

Course.—December 3, the patient had pain in her shoulder when she moved her head. There was bronchial breathing all over the collapsed side. This side moved a little in the upper anterior quadrant, but not below. The posterior

Fig. 32 (Case 5).—Six months after operation. Retraction of left side of chest.

edge of the scapula lay considerably in front of and medial to the medial fragments of the ribs. She was allowed to sit up one-half hour a day, which caused no increase of sputum. Jan. 17, 1922, she had otitis media.

February 16: In the posterior axillary line, at about the fifth rib, there was amphoric breathing with a few distant moist râles. The left lung was well collapsed, though part of the cavity probably persisted. The patient was generally much improved (Clark).

TABLE 5.—Summary of Data in Case 5

	Temperature, F.	Pulse	Respiration	Sputum, C.c.	Weight, Pounds
Before operation, Nov. 1, 1921	98-99.5, occasionally to 100	80-100	25	98, daily regularly	116
Two weeks after operation	98.6-100	100-110	23	10	?
December	98.6-99.5	90-100	25	5	108
January, 1922	97 -98.6	85-110	30	5-10	112<115
Present	Normal	Normal	Normal	About 4	124

Urine negative.
Blood: Hemoglobin, 80 per cent.; red blood cells, 4,500,000; white blood cells, 11,800; polymorphonuclears, 75 per cent.; small lymphocytes, 15 per cent.; large lymphocytes, 10 per cent.; transitionals, 1 per cent.; eosinophils, 1 per cent.

TABLE I.—Summary of Data of Five Cases of Thoracoplastic Compression

	Patient	Involvement	Pneumothorax	Weight		Temperature		Sputum		Tb. bacilli		Vital Capacity			
				Before	After	Before	After	Before	After	Before	After	Before	After	Cured?	Improved
1.	Mrs. M. M.	Cavity left apex; slight involvement right apex	Unsuccessful	115	120¾	98-100	97-98.6	25-30	0	+	—	?	?	+	..
2.	Miss E. W.	Cavity right; serous pleurisy with adhesions	12 attempts; partial success	176	162	98-99	98-99	50-100	10-12	+	—	?	?	+	..
3.	Miss T. T.	Right, 3 cavities; slight involvement left apex	3 attempts unsuccessful, 1 attempt 75 c.c.	130	125	98-100	98-99	40	1st 20 2d 17.5 3d 16	+	+	?	1st 1,550 2d 1,400 3d ?	..	+
4.	Miss K. O.	Large cavity upper right; pyopneumothorax lower right	Pyopneumothorax lower chest does not influence cavity	135	133	97-99.5	97-98.6	140	1st 35<120 after influenza 2d 40<120	+	+	?	1st ? 2d 1,250		+
5.	Miss B. N.	Diffuse fibrosis with cavity left apex; calcified spots right hilus; infiltrate right top	3 attempts unsuccessful	116	124	98.6-100	97-98.6	28	4	+	—	1,800	1,350	+	

Note: November, 1922. Apparent cure of Cases 1, 2 and 5; continued improvement of Cases 3 and 4.

February 17, the roentgen ray showed no respiratory excursion of the left ribs. The left diaphragm moved with the right, but with a markedly diminished excursion. The right lung was clear, except for calcified small spots. The mediastinal contents were displaced to the left and did not move even on forced respiration (Chamberlain).

February 25, the patient had a sore throat and headache.

March 16, she was discharged to her home.

Wassermann negative.

The sputum showed tubercle bacilli Nov. 1, 1921. Five examinations in July, 1922, were negative.

Spirometer: Before operation, 1,800 c.c.; after operation, 1,350 c.c.

July, 1922: The patient has been up for about six hours a day, has gained 16 pounds in weight, has good color, and feels strong and well. She still has about 4 c.c. of watery sputum a day, which on five examinations contained no tubercle bacilli. She is probably cured.

It is too early to judge of definite results. This much, however, can be said. None of the five patients has been harmed. All of them have been improved, and their sputum has diminished. Three seem to be cured.

CONCLUSIONS

1. Operative collapse of a tuberculous lung is indicated in mainly unilateral tuberculosis, when an artificial pneumothorax is indicated but cannot be carried out effectively.

2. Chronic fibrous phthisis, with thick pleural deposits and a rigid chest wall, offers the most frequent indication.

3. Operation should be decided on only after close observation and study of each patient, after repeated consultations with a competent internist, and after attempts at compression by means of pneumothorax have failed.

4. The procedure of choice is (*a*) Sauerbruch's total thoracoplasty; or (*b*) partial pneumothorax with partial thoracoplasty.

5. Operation should be done under local anesthesia.

6. Some patients will be cured by operative collapse after other treatment has failed.

QUANTITATIVE STUDIES IN SYPHILIS FROM A CLINICAL AND BIOLOGIC POINT OF VIEW

II. NORMAL ARSENIC *

JOHN A. FORDYCE, M.D.; ISADORE ROSEN, M.D.,
AND
C. N. MYERS, PH.D.
NEW YORK

In the previous article [1] we described in detail a method of determining quantitatively minute amounts of arsenic in biologic material. In order that a conclusion not influenced by a normal variation can be obtained from this series of analyses, it has been necessary to make a study of the possible arsenic content both in patients who are receiving and in those who have never had any treatment whatever in which arsenic is used as a remedial agent.

The ingestion of foods prepared by artificial processes, fruits which may have been sprayed with arsenical solutions, sea food which in some localities is contaminated to a large degree with arsenic, copper, etc., candy made from glucose, together with a large variety of other possibilities of arsenic ingestion, makes it necessary to gain some idea as to the "normal" arsenic content among our patients. Gautier and Clausmann [2] analyzed a variety of food articles, meats, milk, eggs, fish, shellfish, vegetables, wines, from whose arsenic content they estimate that each inhabitant of Paris receives 7.66 mg. arsenic per annum in his food. Water supply and air also contribute to the possibilities of the presence of arsenic. Brewers quite generally used glucose, which probably was responsible, for some, at least, of the cases of "arsenuria." Arsenic is also occasionally found in baking soda, glycerin, syrups, magnesium sulphate (Epsom salts), sodium sulphate, (Glauber's salt), bismuth subnitrate, and other medicinal products. We also have the

* From the Department of Dermatology and Syphilology and the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, New York.

1. Fordyce, J. A.; Rosen, I., and Myers, C. N.: Quantitative Studies in Syphilis from a Clinical and Biologic Point of View, Am. J. M. Sc. **164**: 242, 1922.

2. Gautier, A.: Arsenic dans des eaux de mer, dans le sel gemme, le sel de cuisine, les eaux minerales, etc. Son dosage dans quelque reactifs usuels, Compt. rend. **137**:232, 1903. L'arsenic existe-t-il dans tous les organes de l'economie animal? Compt. rend. **137**:295, 1903. Origines alimentaires de l'arsenic normal chez l'homme, Compt. rend. **139**:101, 1904.

famous beer drinkers' cases in England in 1900. These reports are found in articles by Hehner,[3] Reynolds and the Royal Commission.[4]

These brief references to the possibilities of finding arsenic in the patients in this locality make it imperative to give some attention to the proper control of normal arsenic content.

A short historical reference to the normal arsenic content will clarify some of the findings which are obtained when only a casual study is made.

Couerbe[5] announced to the French Academy that he had found traces of arsenic in the putrid bodies of human beings. Orfila,[6] Devergie, and others (1838-1840) energetically defended the statement that arsenic was a normal constituent of human bones. In 1841, Orfila and Devergie abandoned their belief in "normal" arsenic as a result of the denial of the truth of these views by Flandin[7] and Danger,[8] as well as recognizing as a result of further analyses that their previous determinations were affected by error. This error emphasizes the necessity for great care in selecting very pure reagents, a fact that has been given very considerable thought throughout this entire investigation. Devergie attributed his findings to the existence of traces of arsenic in his reagents and to the great delicacy of the Marsh test. Orfila regarded it in the light of "history of the so-called normal arsenic as an impenetrable mystery."

The question then lay dormant until 1899 when Gautier[9] presented a series of papers to the French Academy in which he claimed that arsenic is a normal and consistent constituent of the thyroid, mammary glands, brain, thymus, hair, skin, milk and bones of man and of herbivorous and carnivorous animals. The amounts present in the various organs were given as follows: thyroid, 0.76 mg. in 100 gm. tissue; mammary gland, 0.13 mg. in 100 gm. tissue; brain, variable quantity, or none. Tissues from hog, sheep and man were examined. In the

3. Hehner, O.: Discussion on the Occurrence and Detection of Arsenic in Manufactured Products, J. Soc. Chem. Ind. **20**:189, 1901.

4. Royal Commission: The Royal Commission on Arsenical Poisoning, Brit. M. J. **2**:1483, 1557, 1610, 1903. The Final Report of the Royal Commission on Arsenical Poisoning, Lancet **2**:1674, 1746, 1903.

5. Couerbe, J. P.: Du cerveau consideré sous le point de vue chimique et physiologigue. Paris, E. J. Bailly et Cie., Reprint from Ann. de chim. et phys. **56**:1934, 1834.

6. Orfila, P.: Traite des poisons tirés des regnes nineral, vegetal, et animal, ou toxicologie generale, considerée sous les rapports de la physiologie, de la pathologie et de la medecine legale, Paris, 1852, Labe, 5 de.

7. Flandin, C.: Traite des poisons, ou toxicologie applique a la medecine legale, a la physiologie et a la therapeutique, Paris, Bachelier, 1846-1853.

8. Danger, E. P., and Flandin, C.: De la localization des poisons. Note adresée a l'Academie des sciences en reponse a un article de M. Orfila, Paris, 1844.

9. Gautier, A.: Sur l'existence normale de l'arsenic chez les animaux, et sa localisation dans certains organes, Compt. rend. **129**:929, 1899.

normal blood Gautier believes the amount does not exceed 1 : 50,000,000 or 0.00000002 gm. metallic arsenic. Bertrand,[10] Schaefer,[11] Pagel,[12] Knecht and Dearden[13] and Segale (1904) confirmed the results of Gautier.

The translator of "Detection of Poisons" by Autenrieth (1921) having carefully examined the literature also regarded the subject of sufficient importance to discuss it to considerable extent. Following Gautier's report to the French Academy, a committee of that body examined the existing results and arrived at a conclusion as follows:

> Speaking from a medicolegal point of view, I would state that arsenic, aside from the thyroid, mammary and thymus glands, never occurs in the human body except in the skin, hair, bones, milk and sometimes in the feces and then only in traces which are often infinitesimal. Excepting the brain, the other organs and fluids, especially those forming the bulk of the body, as muscular tissue, liver, spleen, kidneys, lungs, blood, urine, etc., fail to show the slightest trace of arsenic. . . . If a chemist therefore examines individually these arsenic-free organs by my method or by one less delicate and finds traces, especially appreciable traces, of this metalloid, such arsenic has been absorbed during life either medicinally or criminally.

Observer	Material	Arsenic Found	Remarks and Conclusions
Gautier	Human and animal organs and other material	0.75 mg. in 100 gm. of human thyroid gland	Used six glands and assumed uniform distribution of arsenic
Bertrand	Only animal material	0.015 mg. per 100 gm. of dried sponge	Concludes arsenic is a normal constituent of protoplasm
Schaefer	Human organs	0.007 mg. per 100 gm. of human thyroid	Concludes arsenic may occur in all organs but found many free from arsenic
Pagel	Human and animal organs	Positive but not quantitative	Found testes arsenical but Gautier says they are not

On the contrary, several chemists have carefully analyzed human and animal organs, either finding no arsenic or detecting this metalloid in mere traces, which are inconstant in occurrence and confined to no special organ. The table on this page briefly summarizes their results.

The results set forth in this table place "normal arsenic" in a doubtful position at least. If it is a reality and not a fancy, the quantity of arsenic, compared with that obtained in an analysis actually dealing with this metalloid, is so minute that the toxicologist need feel no concern. If he has con-

10. Bertrand, G.: Sur l'existence de l'arsenic dans la serie animale, Bull. de la Soc. Chim. de Par. **27**:1233, 1902; Compt. rend. **135**:809, 1902. Sur l'existence de l'arsenic dans l'organisme, Compt. rend. **134**:1434, 1902. Nouvelles Recherches sur l'arsenic de l'organisme. Presence de ce Metalloide dans la Serie Animale, Ann. de l'Inst. Pasteur **17**:1, 1903. Sur l'existence de l'arsenic dans l'oeuf de la poule, Compt. rend. **136**:1083, 1903. Emploi de la bombe calorimetrique pour demontrer l'existence de l'arsenic dans l'organism, Compt. rend. **137**:266, 1903.

11. Schaefer, G.: Recherches sur l'existence normale de l'arsenic dans l'organisme humain, Ann. chim. anal. **11-12**:52, 97, 1906.

12. Pagel, J. L.: Dissertation, 1900, University of Nancy.

13. Knecht, E., and Dearden, W. I.: The Elimination of Arsenic Through the Hair and Its Relation to Arsenical Poisoning, Lancet **1**:854, 1901.

ducted his analysis with every precaution as regards reagents and method and obtained a distinct mirror, he may dismiss the "normal arsenic" chimera and accept the result as due to arsenic that has entered the body from some external source. Kunkel has summed up the matter in these words.

"The so-called normal arsenic, if there is such a thing, does not effect the results of forensic chemistry, because the so-called normal quantities are so exceedingly small (0.01 or even 0.001 mg. in an organ) that the quantities necessary to furnish a satisfactory forensic proof, which are a hundred or even a thousand times greater, must be regarded as an entirely different and much higher order of magnitudes."

In direct contradiction to the results of Gautier, Ziemke[14] obtained contradictory results.

In discussing the divergence between his results and those of Gautier, Ziemke considers that there can be no error of manipulation or question of the purity of reagents in the work of the French toxicologist, because of the uniformly negative results which he obtained with organs other than those mentioned; and he attributes the difference in the results attained, firstly, to the known fact that certain organs take up arsenic more readily and retain it more persistently than others, and, secondly, to the fact that in Germany, under existing laws, articles of food, condiments, etc., are subject to a very strict supervision, and that the possibility of the introduction of arsenic into the system by this means is very slight, while in other countries arsenic is frequently found in articles used in the household and also in food products. He cites the statement of Shattuck that in the United States the urine of many healthy persons contains arsenic; which, according to Richter is not the case in Germany. This may account for the presence of arsenic in the thyroids of man, or even of the dog and hog, but hardly for that found in the sheep.

This statement is attributed to Shattuck by many writers, particularly among the Germans. It is, however, not original with him, but is a quotation from Putnam (Boston M. & S. J. **122**:421, 1890), who states that of forty-eight samples of urine collected at random from the outpatients at the Massachusetts General Hospital, twenty-one were found to contain traces of arsenic. Shattuck's paper is the report of an address favoring the passage of legislation in Massachusetts to restrict the use of arsenical pigments in the manufacture of wall papers, and Putnam's observations were apparently prompted by the same interest, and although he states that these patients "did not present any symptoms which seemed referable to arsenical poisoning," they were certainly not "healthy," and there is no assurance that they had not been exposed to arsenical contamination. One of the alternatives suggested by Abel and Buttenberg (Ztschr. f. Hyg. **32**:478, 1899), in referring to Shattuck, namely, that "people in America deal carelessly with arsenic," would very probably have been accepted by both Shattuck and Putnam as a more reasonable explanation of the presence of arsenic in these urines, than the hypothesis of "normal arsenic," which was not entertained at that time.

There is no irreconcilable contradiction between these apparently inconsistent results from a medicolegal point of view, although there may be from that of physiology. Gautier's supposition that arsenic is a "normal" element of certain tissue constituents, and plays a part in their functioning, like the iodin in the thyroid, is clearly negatived if it be shown not to be a constant constituent of those tissues. That question is one of notable interest to the biological chemist, but it does not concern the toxicologist, to whom it is immaterial whether arsenic found in these minute quantities with great

14. Ziemke, E.: Ueber Vorkommen von Arsen in menschlichen Organen und seinen Nachweis auf biologischem Wege, Viertljschr. f. ger. Med. **23**: 51, 1902.

frequency in certain organs, not usually submitted to analysis in forensic cases, is normal or of fortuitous origin.

There can be no doubt, however, that arsenic is very widely distributed in nature and is constantly taken into the human economy in very minute quantities. Gautier has found that sea salt contains from 0.001 to 0.045 mg. arsenic in 100 gm., and rock salt from 0.0025 to 0.014 mg. Sometimes these arsenic values are of such nature as to affect entire communities, as when the water supply or air contains arsenic. Or certain individuals only in a community may absorb arsenic from food articles more highly arsenical than those referred to above, as in the Manchester beer poisonings. Several investigations in which delicate and direct methods of examination were used, and the purity of the chemicals was assured, have shown that the presence of arsenic in human urine, in quantities very rarely exceeding 0.1 mg. per liter, and in the absence of any history of its administration, is by no means unusual, at least in Boston and its vicinity; and we have no reason for supposing that conditions differ there in this regard from what they are in other cities in the United States. Putnam found arsenic in 30 per cent. of 150 samples of urine examined. Sanger detected arsenic in twenty cases, and Hills, examining 260 samples from 180 patients, found arsenic in 135, or 75 per cent. In five of these cases Hills found exceptionally as much as from 0.3 to 0.5 mg. per liter. In many of the cases the origin of the arsenic was traceable to arsenical wall hangings, etc., but in others it could not be so accounted for. Hills suggests that the very general use of Paris green as an insecticide, may have produced an arsenical impregnation of the soil in certain localities, from which growing vegetables might become contaminated.

Gautier and Clausmann [15] studied the methods of destroying organic matter, and Gautier [16] revised some of the results published in 1899 [9] and 1903 [3] with reference to the quantity of arsenic in normal tissues and thinks that the error was due to traces of arsine in the hydrogen sulphid which was used for precipitating the arsenic. In this paper, the results of Gautier's previous experiments are reviewed in relation to the traces of arsenic which had been previously discovered in tissues. In his former investigations, the quantities of arsenic which had been reported were a trifle too large, due to the error which has just been mentioned. The mention of this fact does not modify essentially the first conclusions on normal arsenic, its localization and mode of elimination, but has resulted in a revision of the method of destruction of tissues used in medicolegal experiments. In conclusion, Gautier points out that it should be proved beforehand that there are no detectable quantities of arsenic in the nitric, sulphuric, and hydrochloric acids used.

Bloemendal [17] found that normal urine contained traces of arsenic and that traces may be found under normal conditions in the body of

15. Gautier, A., and Clausmann: A New Method for the Destruction of Tissues for the Detection of Arsenic and the Examination of Their Ashes, Compt. rend. **165**:11, 1917.

16. Gautier, A.: On Normal Arsenic in Living Tissues, and Traces of Iodine Found in Air and Water, Compt. rend. **170**:261, 1920.

17. Bloemendal, W. H.: Arsenic in the Animal Organism, Arch. Pharm. **246**:599, 1909. Arsenical Poisoning in Vineyards, Brit. M. J. **2**:86 (July 16) 1921.

man and animals but that it has no physiologic significance. As a result of his work he concluded that arsenic does not pass into the foetal circulation.

Bang [18] found arsenic in normal urine in twenty-five cases in daily amounts up to 0.5 mg. Only in three cases was arsenic not found. The greatest amount of arsenic in the urine was found after a fish diet. During the use of a vegetable and milk diet, the urine is usually free from arsenic.

Klason [19] draws the conclusion that arsenic is a normal constituent of the human body. Klason examined the urine of a man who had never taken arsenic in any form, nor had he suffered from "arsenic sickness." He found that the urine of this man always contained arsenic to the extent of from 0.005 to 0.0125 mg. per liter. Klason states that the methods which have hitherto been used for the estimation of arsenic in organic secretions are so inexact that the results obtained are open to criticism.

McNally [20] investigated the retention of arsenic in two cases in which arsenic had been administered. The results are expressed in grams of arsenous oxid in 100 grams of the tissue. In the first case the man died four and one-half hours after taking an unknown amount of some form of arsenic. The amounts are: stomach walls, 0.0328 gm.; stomach contents, 1.1782 gm.; liver, 0.0191 gm.; small intestine, 0.0176 gm.; large intestine, 0.0345 gm.; left kidney, 0.0122 gm.; right kidney, 0.0108 gm.; heart, 0.0085 gm.; pancreas, 0.0125 gm.; lung, 0.0027 gm.; gallbladder, 0.0055 gm.; esophagus, 0.0224 gm.; brain, 0.00026 gm. In the second case the results are on a patient who died ten days after the last dose of an arsenical. The death is reported as typical of arsenical poisoning. The results of examination of tissues for arsenic were as follows: spinal cord, 0.00275 gm.; blood, 0.0066 gm.; stomach wall, 0.00066 gm.; stomach contents, 0.00066 gm.; kidney, 0.00211 gm.; spleen, 0.00171 gm.; liver, 0.00171 gm.; urine, 0.00363 gm.

Generally speaking, conflicting reports, whether positive or negative, are usually based on the quotation "if arsenic were a normal constituent, there could be no exception, and therefore its presence in the cases in which it has been found must be adventitious."

---

18. Bang, I.: The Amount of Arsenic in Normal Urine, Allm. Sv. Läkaretidn **13**:549, 1916. Quantitative Determination of Arsenic in Urine, Schweiz. Apoth. Ztg. **55**:393, 1917.

19. Klason, P.: Quantitative Determination of Arsenic in Organic Secretions and Compounds, Ark. Kemi. Min. Geol. **5**:9, 1914. Determination of Small Amounts of Arsenic and the Normal Arsenic Content of Urine, Svensk Kem. Tidskr. **28**:69, 77-80; Ark. Kemi. Min. Geol. **6**: No. 6, pp. 1-5, 1916. Quantitative Determination of Arsenic in Organic Secretions and Compounds, Ark. Kemi. Min. Geol. **6**:1, 1916.

20. McNally, W. D.: Retention of Arsenic in the Organs, J. Am. Chem. Soc. **39**:826, 1917.

We do not accept nor do we resort to this argument. As pointed out in the beginning of this article, diet was recognized as a source of arsenic and on this basis the history of each patient shows in a general manner the nature of the diet consumed. Direct personal contact with careful records, as given in the data below, has assisted us materially in arriving at the results which are contained in this article.

The results of investigation can only affirm or cast doubt on the presence or absence of arsenic as a normal constituent. In order to control these factors of food and medication satisfactorily, careful histories have been taken on all the patients. More detailed discussion of each table and its clinical aspect will be found later. The discussion of the charts will only refer to the normal arsenic aspect of this series of articles. Control methods have been employed throughout this investigation and conclusions are arrived at only after an extensive examination of a large group of patients so that the errors due to the biologic economy are reduced to a minimum. A one-patient or a two-patient study will add little accurate knowledge with a wide application to others. The composition of some of the drugs used by the patients has been investigated in relation to the arsenic content; likewise glucose, a common article of food in beverages, delicatessen products, ice creams, candy, and bakery products, was examined. The latter proved interesting in its application to a few patients who, it was presumed, were on a sugar-free diet. Three samples of glucose were examined for arsenic and it was found that only the purest preparation was free of arsenic. Sample 2 contained 0.778 mg. arsenic per hundred grams, and sample 3 contained 0.10 mg. arsenic per hundred grams. These figures found ready application to a series of patients using food products containing glucose syrups and bakery supplies as well as to candy eaters who indulged to a considerable extent. Glycerol, a constituent of some rhubarb and soda, is another important product to be considered. The other drugs will be noted in a table showing the remedies used.

RESULTS OF EXAMINATION

Normal Hair.—Hair from twenty-one persons, fifteen men and six women, was examined for arsenic. In nine specimens no arsenic was detected; in twelve arsenic in amounts varying from 0.052 to 10.6 mg. was found. In the cases showing the smaller quantities no definite data were obtainable to determine the source of the arsenic, that is, whether any preparation had been employed on the scalp or whether ingestion, environment, occupation, etc., were responsible.

Specimens 1722 and 1723 were from husband and wife, the larger amount being from the latter. Investigation proved that both were using a hair tonic in which arsenic was a contamination of one of the ingredients. The blood and urine in these cases were arsenic free.

TABLE 1.—Arsenic in Normal Hair

Patient Serial No.	Weight of Specimen, Dry	Arsenic in Mg. per 100 Gm. of Specimen	Patient Serial No.	Weight of Specimen, Dry	Arsenic in Mg. per 100 Gm. of Specimen
1612	1.3427	0.992	1723	0.1944	3.08
1629	3.0806	None	1627	0.4634	None
1614	2.5777	None	1610	1.6476	10.6
1720	0.8428	0.0593	1628	1.7961	0.280
1630	0.5641	None	1496	1.1003	None
1725	0.7362	None	1626	0.6561	None
1721	1.9112	0.0523	1613	3.1559	0.950
1611	1.7902	None	1731	0.2903	0.166
1496	1.7685	0.452	1783	1.6400	0.180
1497	0.5500	None	1816	1.5984	0.063
1722	1.2427	2.14			

Specimen 1610, with 10.6 mg. arsenic, can be traced directly to contact with arsenic, this hair being obtained from one of the chief chemists of a large drug manufacturing concern whose work was largely connected with the arsenicals. Specimens 1496, 1612, 1613, 1628 and 1731 were also from persons who handle arsenicals. These people all had a thorough shampoo before the hair was obtained so that no mechanical contamination was responsible for the presence of arsenic.

Generally speaking, the hair may contain very small quantities of arsenic but it is questionable if it will be found in more than 50 per cent. of persons unless medication plays an important part.

TABLE 2.—Arsenic in Normal Human Milk

Patient Serial No.	Weight of Specimen		Percentage Solids	Arsenic in Mg. per 100 Gm. of Dry Specimen
	Moist	Dry		
1654	6.4550	0.7798	12.08	None
1656	6.9065	0.9313	13.48	None
1648	4.4997	0.5531	12.31	None
1650	5.8792	0.9025	15.35	None
1652	6.6156	0.9261	13.99	None
1657	6.4643	0.9152	14.18	None
1659	6.2586	0.7312	11.68	None
1655	5.1438	0.5346	10.39	0.9
1660	6.2221	0.8489	13.64	0.6
1658	3.2825	0.3650	11.11	None
1651	7.4236	0.9491	12.78	None
1662	4.8614	0.4982	10.24	None
1653	5.3512	0.6885	12.86	None
1649	5.3968	1.5886	10.91	None
1661	4.4750	0.5519	12.33	None

Human Milk.—It has been shown by several investigators that arsenic when taken in large doses may be secreted in milk. The milk examined in this study was obtained from fifteen nursing mothers in a maternity hospital. They were all healthy women on a mixed hospital

diet of meat, vegetables, cereals and the usual fluids. In thirteen cases no arsenic was demonstrable; in two cases (Nos. 1655 and 1660) it was present in amounts of 0.9 and 0.6 mg., respectively. An attempt to determine whether these patients had been taking tonics containing arsenic was unsuccessful.

RESULTS OF EXAMINATION

Normal Urine.—These specimens were obtained from persons who denied syphilis or the use of any arsenicals. Eighteen specimens were examined, from sixteen men, fifteen white and one black, and two women, one white and one black. The ages of these persons varied from 16 to 68 years. As to occupation: two were chemists, two were students, one was a pharmacist and thirteen were not engaged in any particular line of work.

TABLE 3.—ARSENIC IN NORMAL URINE

Patient Serial No.	Weight of Specimen		Percentage Solids	Specific Gravity	Arsenic in Mg. per 100 Gm. of Specimen	
	Moist	Dry			Moist	Dry
1480	29.9702	0.5067	1.99	1.015	0.00556	0.278
1131	32.3625	0.9525	2.94	1.018	0.525	17.8
1435	32.0655	1.2091	3.77	1.022	None	None
1140	35.01	0.749	2.14	1.012	None	None
1135	32.243	1.6721	5.03	1.126	0.0625	1.04
1438	32.2200	1.2136	3.76	1.028	0.00931	0.247
1437	32.2425	1.6931	5.25	1.031	0.049	0.945
1310	34.3300	0.8793	2.55	1.016	0.00875	0.34
1481	30.9418	1.7116	5.53	1.037	0.172	3.11
1200	32.0655	1.4783	4.61	1.028	0.00311	0.067
1204	3.134	0.6215	2.00	1.015	0.01064	0.535
1205	31.7606	1.2056	3.80	1.026	None	None
1482	30.9060	1.2838	4.15	.....	None	None
1308	13.1725	1.4698	4.42	1.021	0.0129	0.290
1478	29.7205	0.8481	2.85	1.020	0.0448	1.57
1477	30.6655	1.1484	3.74	1.025	None	None
1479	32.9219	1.7238	5.23	1.030	None	None
1215	32.2435	1.1044	3.43	1.020	0.0036	0.106
1214	32.4375	0.5003	1.54	1.010	0.00306	0.19

Clinical investigation showed that fourteen of these patients were in normal health, that three were tertiary syphilitics and one a primary syphilitic. The tertiary syphilis patients were apparently truthful in stating that they had received no arsenical treatment, for arsenic was not present in appreciable amounts in their urine. The primary syphilis patient, a young man, aged 21, weighing 115 pounds, admitted exposure four weeks previously and was found to have a chancre on the shaft of the penis behind the corona, in which treponemas were demonstrated by the dark field examination. His Wassermann reaction was negative. His diet consisted of fish once a week, red meats on the other six days, potatoes twice daily, with other vegetables, eggs and milk in moderation; candy and pastry occasionally. His urine on analysis showed 17.8 mg. of metallic arsenic. From this large amount we can only infer that the patient deliberately withheld the truth regarding previous treatment for his infection and that he must have received an intra-

venous injection of one of the arsenicals elsewhere before coming to our clinic. The presence of so great an amount could be accounted for only in this way.

Three positive results (Nos. 1481, 1437 and 1135), the quantities of arsenic varying from 0.67 to 3.11 mg., can be accounted for on dietary grounds. This was nicely illustrated in two cases (Nos. 1481 and 1478), two youths, who when the specimens were taken denied having eaten candy or other sweets. When confronted with the results of the examination, however, they both admitted having eaten unusually large amounts of candy for several days prior to the tests. Candy is made largely from glucose which contains varying amounts of arsenic, depending on the quality.

TABLE 4.—Arsenic in Normal Urine

Patient Serial No.	Weight of Specimen		Percentage Solids	Specific Gravity	Arsenic in Mg. per 100 Gm. of Specimen	
	Moist	Dry			Moist	Dry
1196	32.15	0.2986	0.92	1.010	0.00155	0.16
1306	31.0065	1.4230	4.56	1.023	None	None
1212	32.8334	1.3572	4.14	1.022	0.0031	0.73
1311	35.6495	1.3140	3.68	1.017	0.042	4.4
1312	31.2630	1.2765	4.08	1.021	0.048	1.1
1307	31.3400	1.4810	4.75	1.023	0.0032	0.067
1301	30.6655	1.2790	4.17	1.031	0.0065	0.15
1309	29.7205	1.1965	4.02	1.021	0.027	0.669
1132	34.06	1.552	4.56	1.027	0.00298	0.064
1302	35.2982	0.6007	1.701	1.011	None	None
1317	12.9700	0.2164	1.66	.....	0.123	1.39
1195	32.883	1.5658	4.79	1.028	0.0121	0.255
1197	32.0337	2.0312	6.34	1.033	None	None
1210	30.9785	1.4885	4.81	1.023	0.0193	0.403
1304	31.9271	0.5486	1.71	1.012	0.0016	0.0929
1134	32.4375	2.0249	6.21	1.031	0.00154	0.024
1303	35.330	1.3527	3.82	1.026	0.100	2.5
1136	30.9418	1.0695	3.46	1.921	0.00161	0.0467
1202	31.6773	2.2397	7.07	1.038	0.0526	0.744
1199	32.7995	0.8421	2.59	1.021	None	None
1203	31.3858	0.7203	2.29	1.015	0.4246	18.5
1322	32.2435	1.3065	4.05	1.027	None	None

Normal Urine and Blood.—In this series of nineteen cases a comparison was made of the amounts of arsenic found in urine and blood taken simultaneously.

There were thirteen men—eleven white, two black—and six women—four white and two black. Their ages ranged from 20 to 55 years. Occupations included four houseworkers, a machinist, an actor, a dressmaker, a shipping clerk, a chauffeur, a tailor, an elevater operator. Five subjects had no occupation.

These patients were selected in the skin clinic after affirming that they had taken no drugs or medicine of any description. This point was especially emphasized as subjects admitting medication were placed in another group. The untrustworthiness of patients' statements, however, is demonstrated in some of the results charted.

The clinical classification is as follows: Ten cases of tertiary syphilis with a positive Wassermann only; one case each of early secondary syphilis, seborrhoeic dermatitis, chromophytosis, pityriasis rosea, sycosis vulgaris, alopecia areata and mucous cysts of the mouth.

In the case of secondary syphilis, a white woman, aged 30, dressmaker, there was a generalized circinate papular eruption with leukoderma colli, alopecia of the eyebrows and a generalized adenopathy.

TABLE 5.—Arsenic in Normal Blood

Patient Serial No.	Weight of Specimen		Percentage Solids	Arsenic in Mg. per 100 Gm. of Specimen	
	Moist	Dry		Moist	Dry
1247	5.7525	1.6580	28.82	0.00869	0.0301
1284	6.1366	1.3701	22.32	0.050	0.21
1328	4.7426	1.2222	25.77	.....	.....
1289	6.0801	1.3051	21.46	0.0656	0.306
1290	4.6542	0.9413	20.22	0.425	2.12
1285	5.282	1.3838	26.20	0.943	3.6
1099	5.3218	1.1802	22.17	None	None
1288	5.9690	1.6707	28.00	None	None
1287	6.311	1.532	24.28	Trace	Trace
1194	5.8835	1.4086	25.47	0.41	1.6
1241	4.3486	1.1421	26.26	0.269	1.02
1245	5.9133	1.5125	25.58	0.0169	0.066
1286	5.368	1.0405	19.39	0.185	0.96
1103	9.1200	2.3902	26.20	0.022	0.089
1291	5.085	1.4402	28.32	0.039	0.138
1105	6.8532	1.3916	20.35	None	None
1192	5.853	1.2173	20.80	None	None
1246	5.4072	1.2926	23.95	None	None
1175	5.4075	1.2182	22.53	0.185	0.82
1334	4.0060	0.9294	20.70	0.025	0.107

She gave no history of exposure, no evidence of chancre was present, and she denied having had any antecedent treatment. She was on a meat-free diet, eating fruit and vegetables in large quantity. Her blood showed 1.6 mg. arsenic and her urine 0.255 mg. These amounts are excessive and immediately raise the question as to the accuracy of her statement regarding treatment.

Then ten cases of tertiary syphilitics included in Tables 4 and 5 gave the following analysis:

TABLE 6.—Arsenic in Blood and Urine of Ten Tertiary Syphilitics

No.	Arsenic in Blood, Mg.	Arsenic in Urine, Mg.	Source
1	None	0.744	Probably dietary
2	None	0.04	Probably dietary
3	0.08	0.16	Probably dietary
4	0.107	None	Probably dietary
5	0.069	0.024	Probably dietary
6	0.138	2.5	Probably arsenical medication
7	0.066	0.408	Probably dietary
8	0.8	18.5	This patient undoubtedly received arsenical treatment
9	0.9	0.009	Patient was on mixed diet, using large quantities of sweets and sugar daily
10	1.02	None	Probably dietary

TABLE 7.—Arsenic in Normal Blood*

A					B			
Patient Serial No.	Weight of Specimen		Percentage Solids	Arsenic in Mg. per 100 Gm. of Dry Specimen	Diseases	Duration	Amount of Arsenic, Mg.	Remarks
	Moist	Dry						
1935	2.5624	0.6575	25.68	Trace	Acne indurata	4 years	None	
1938	4.8494	1.2473	25.72	0.0400	Acne vulgaris	2 years	None	
1964	2.7822	0.5960	21.42	None	Acne vulgaris	3 years	None	
1960	4.4432	0.9145	20.58	None	Dermatophytosis	4 weeks	None	
1963	3.8308	0.7602	20.00	None	Dermatophytosis	1 year	None	
1939	5.6574	1.0935	19.33	None	Dermatophytosis	1 year	None	
1937	4.2964	0.9658	22.49	0.05	Dermatitis venenata	1 week	None	
1946	3.0083	0.7388	24.55	None	Eczema	6 years	None	
1954	3.8925	0.9546	24.52	None	Epithelioma	8 years	None	
1957	2.6523	0.5652	21.30	None	Furunculosis	1½ years	None	
1961	2.8608	0.6514	22.70	None	Impetigo	1 week	None	
1953	3.8300	0.8124	21.21	None	Keloid	8 years	None	
1965	3.9855	0.9276	23.27	None	Leucoderma	2 months	None	
1944	3.7894	0.8173	21.56	None	Pruritis	2 weeks	None	
1945	3.4509	0.8392	24.31	0.474	Psoriasis	5 years	None	
1962	5.0276	1.4404	25.59	0.0694	Psoriasis	2 years	None	
1932	5.6127	1.3470	24.01	Trace	Psoriasis	18 years	None	
1959	3.4900	0.8152	23.35	None	Seborrheic dermatitis	7 years	None	
1933	4.4975	0.9014	20.31	0.220	Seborrheic dermatitis	1 month	None	
1955	3.2455	0.8936	27.53	None	Seborrheic dermatitis	4 years	None	
1941	2.3392	0.5160	22.05	None	Scabies	1 month	None	
1940	4.4848	1.3412	29.90	None	Syphilis, secondary	4 months	None	
1942	4.5052	1.0412	23.11	None	Syphilis, tertiary	Denied	None	
1943	4.1659	0.9945	23.87	None	Syphilis, tertiary	Denied	None	
1934	4.2633	1.1351	26.65	None	Syphilis (?)	Denied	None	
1936	5.6778	1.4574	25.66	None	Syphilis (?)	Denied	None	
1956	2.0444	0.4390	20.14	None	Syphilis (?)	Denied	None	
1952	3.9001	1.0780	27.64	None	Tinea profunda	5 months	None	
1958	2.9474	0.6849	23.57	None	Tinea circinata	1 week	None	
1951	4.8242	1.2069	25.03	None	Tinea versicolor	1 year	None	

2053	4.4812	0.9902	23.66	Trace	Tuberculosis verrucosa cutis..	1 year	None	
2050	4.0197	0.9049	22.51	0.5525	Urticaria.....	2 weeks	None	
2060	4.4462	0.9856	22.12	0.3378	Urticaria.....	1 week	None	
2062	4.6021	0.9924	21.56	None	Xanthoma.....	3 months	None	
2013	2.8525	0.6930	23.22	None	Tinea.....	3 weeks	Trace	Dietary (?)
2039	3.6004	0.7910	21.60	0.2528	Tinea.....	2 weeks	Trace	Dietary (?)
2061	4.7224	0.7908	16.74	None	Leucoderma.....	8 months	Trace	Dietary (?)
2040	3.5645	0.7445	20.88	0.1343	Syphilis (?).....	Denied	Trace	Dietary (?)
2047	4.4880	1.2329	27.46	None	Pott's disease.....	1 year	Trace	Dietary (?)
2042	4.0151	0.9037	22.50	0.0553	Alopecia areata.....	2 years	Trace	Using hair tonic
2043	3.2021	0.7395	23.09	None	Syphilis (?).....	Denied	0.0597	Dietary (?)
2012	3.3823	0.8834	23.13	0.1098	Dermatitis venenata.....	6 months	0.04	Using calamine and zinc lotion
2049	5.6073	1.4358	25.58	None	Scabies.....	2 weeks	0.05	Dietary
2048	3.2715	0.6589	20.14	Trace	Syphilis (?).....	Denied	0.0503	Dietary
2059	5.7283	1.2587	21.97	0.0397	Folliculitis.....	7 years	0.0553	Using calamine and zinc lotion
2011	4.4076	1.0403	23.60	0.0800	Dermatophytosis.....	3 months	0.0694	Dietary (?)
2015	4.3772	0.9932	22.69	0.0503	Syphilis (?).....	Denied	0.080	Dietary (?)
2041	4.4941	0.9362	20.84	None	Alopecia.....	3 months	0.0662	Using hair tonic
2052	4.5431	1.0142	22.32	0.0986	Syphilis.....	Denied	0.1343	Using calamine and zinc lotion
2058	3.7555	0.7668	20.41	None	Pruritis.....	1 week	0.1668	Using calamine and zinc lotion
2001	3.4480	0.7304	21.18	None	Tinea cruris.....	1 year	0.220	Using calamine and zinc lotion
2045	4.3155	1.0241	23.73	Trace	Impetigo.....	2 weeks	0.2528	Dietary
2014	2.2985	0.5479	23.83	None	Eczema.....	Unknown	0.3378	Using calamine and zinc lotion
2046	4.3005	1.0119	23.75	None	Sudamina.....	2 weeks	0.474	Dietary (?)
2044	2.2678	0.4315	19.02	Trace	Acne.....	5 years	0.5525	Taking R. & S. and using lotio alba

Location of above diseases: Scalp, 2 cases; face, 11; neck, 2; chest, 2; trunk, 3; spine, 1; upper extremity, 6; hands, 9; lower extremity, 6; feet, 5; general, 8.

* Tables 7 and 8 contain the detailed analyses showing the disease, duration of disease, amount of arsenic found, with remarks as to the possible cause of the presence of arsenic. Findings in patients having no arsenic in their blood are arranged in that order in the second part of the table. For example, patient 1964 had no arsenic in the blood, had acne indurata for four years, etc. Patient 1935 had tinae of three weeks' duration with only a trace of arsenic, probably of dietary origin. The results in portion A of each table are amplified by those in portion B in the order of magnitude in which the arsenic in found.

The patient with seborrhoeic dermatitis had 0.21 mg. arsenic in the blood and none in the urine; the one with sycosis vulgaris was negative in both blood and urine; the case of alopecia areata showed a trace in the blood and 1.39 mg. in the urine; chromophytosis, 0.306 mg. in the blood and 0.067 mg. in the urine; pityriasis rosea, 3.60 mg. in the blood and 0.669 mg. in the urine, and the patient with mucous cysts in the mouth had 2.12 mg. in the blood and 0.15 mg. in the urine. The last named was probably taking arsenic internally. In the case of pityriasis rosea the only explanation we could find was that of absorption from various ointments which the patient had applied locally. The source of the arsenic in alopecia areata and chromophytosis was either dietetic or medicinal.

The remaining two patients in this group came for a blood test. One was negative in both blood and urine: the other had 0.73 mg. in his urine; his blood was lost; dietetics or medication would account for his excess.

Normal Blood.—At the outset of this research we did not appreciate the difficulty of obtaining normal blood from absolutely normal persons. Our primary purpose was to obtain such specimens from groups of people, for example, in certain industries, but owing to lack of opportunity this procedure was not found feasible. Therefore, recourse was had to patients coming to the dermatologic department for minor affections of the skin. A special effort was made in the selection so that this series represents patients who came to the clinic for the first time and in whom we could exclude syphilis and, therefore, eliminate the possibility of their having received any arsenical medication.

Our analysis brought out the following facts: 55 cases: 41 men—32 white, 9 black; 14 women—10 white, 4 black. Ages ranged from 15 to 59 years. Occupations: none, 16; chauffeur, 2; sailor, 1; houseworker, 10; electrician, 1; waitress, 1; carpenter, 1; tailor, 5; laborer, 2; student, 3; shoemaker, 1; porter, 3; clerks, 4; teacher, 1; laundryman, 1; painter, 1; mechanic, 1; business man, 1.

Normal Blood.—This group comprises clinic patients, most of them being old dermatologic cases, syphilitic cases who claimed they had had no previous treatment containing arsenic in any form and patients who came simply for a Wassermann examination of their blood.

Seventy-eight specimens were obtained from forty-one men—32 white and 9 black—and thirty-seven women—31 white and 6 black. Their ages ranged from 15 to 59 years. Their occupations were given as follows: None, 25; housework, 24; chauffeur, 1; waiter, 9; laborer, 1; furrier, 1; letter carrier, 1; printer, 1; collector, 1; sailor, 1; candy manufacturer, 1; student, 1; gelatin worker, 1; tailor, 5; shipping clerk, 1; office workers, 3; bookbinder, 1.

Location of diseases enumerated in Table 8: Face and head, 12; neck, 2; mouth, 2; chest, 4; trunk, 4; upper extremity, 2; hands, 4; lower extremity, 3; genitals, 2; eye, 3; nervous system, 1; general, 11; no external lesions, 28.

The data in these cases are summarized in Table 8.

In view of the fact that arsenic was present in so many of the cases under external or internal treatment, we can only assume that it was due to retention when ingested or absorption through the skin when applied locally. Arsenic, as is well known, has a cumulative effect and many of these patients had been using these drugs over a long period. In persons with a diseased skin, absorption is, of course, more active. This has been illustrated a number of times in patients with epitheliomas who developed multiple neuritis after treatment with arsenical paste and died from arsenical poisoning.

The tables show the composite results of analysis together with a chemical interpretation of each table. In order to control these results, the specimens were obtained under uniform conditions and analyzed as described in a preceding paper.

Blood was obtained in sterile Wassermann tubes and weighed as soon as possible after being taken so that as much evaporation as possible was eliminated. The percentage of solids was obtained by evaporation on the water bath and then in the hot air oven at 110 C. to constant weight.

The urine was collected by the patient with no control as to the amounts of liquid ingested. The specimen was weighed as a liquid, then evaporated on the water bath and to constant weight in the hot air oven. Specific gravity determinations were made at 25 C.

In order to control the possibilities of arsenic contamination, the needles used for taking the blood were sterilized in the usual, routine manner. The needles were then washed forcibly with a syringe and distilled water. The water was analyzed for arsenic. Four samples showed none; one sample contained a trace, three samples contained a half part, and one sample contained six parts. These amounts were so small, with one exception, that they would in no way offer an explanation of any arsenic found in normal blood. In Table 6 all the specimens analyzed were obtained by the use of thoroughly cleaned, new needles.

Analysis of the rhubarb and soda mixture showed that it contained 0.0613 mg. arsenic per hundred grams of drug. The calamine lotion analyses were as follows: solids, 17.09, 17.99, 16.58 and 18.12 per cent., respectively, and the corresponding arsenic content per hundred grams

TABLE 8.—Arsenic in Normal Blood

A						B			
Patient Serial No.	Weight of Specimen		Percentage Solids	Arsenic in Mg. per 100 Gm. of Specimen		Diseases	Duration	Amount of Arsenic, Mg.	Remarks
	Moist	Dry		Moist	Dry				
1592	3.9527	0.9062	23.17	1.01	4.41	Acne vulgaris	2 months	None	
1529	3.8685	0.0371	24.08	Trace	Trace	Acne vulgaris	4 years	None	
1598	7.3504	1.5054	20.45	0.407	1.99	Aphthous stomatitis	3 years	None	
1640	6.0826	1.3132	21.58	0.0164	0.1761	Bazin's disease	2 years	None	
1598	5.0975	1.0568	20.77	None	None	Carcinoma breast	18 months	None	
1491	5.1097	1.1635	22.76	None	None	Eczema	Unknown	None	
1643	4.8236	1.1080	22.97	None	None	Eczema	3 weeks	None	
1468	4.7397	1.0180	21.47	0.117	0.560	Eczema	1 year	None	
1101	7.0233	.....	.....	None	None	Epithelioma	Unknown	None	
1608	4.5919	1.1019	23.99	None	None	Iritis	Unknown	None	
1493	5.5765	1.3015	23.33	None	None	Leprosy	Unknown	None	
1646	3.6348	0.9566	26.31	0.0132	0.0522	Onychomycosis	4 months	None	
1060	6.7280	1.6555	24.60	0.03	0.12	Sebaceous cyst	4 months	None	
1637	4.7328	0.9608	20.300	0.158	0.780	Syphilis (?)	Denied	None	
1581	4.5324	0.9548	21.06	0.183	0.873	Syphilis (?)	Denied	None	
1536	4.3641	0.9940	22.77	None	None	Syphilis (?)	Denied	None	
1494	5.5300	2.2673	30.11	0.053	0.17	Syphilis, secondary	4 weeks	None	
1608	5.5355	1.1963	21.61	Trace	Trace	Syphilis, secondary	2 months	None	
1106	5.292	1.2010	22.69	None	None	Syphilis, tertiary	Unknown	None	
1532	5.3238	1.0415	19.56	0.375	1.92	Syphilis, tertiary	Unknown	None	
1545	4.5911	1.0925	23.79	None	None	Syphilis, tertiary	11 years	None	
1582	4.4355	0.9506	21.43	None	None	Syphilis, tertiary	24 years	None	
1530	4.3169	0.9941	23.02	0.0231	0.1005	Syphilis, tertiary	Unknown	None	
1602	5.4417	1.0615	19.50	None	None	Syphilis, tertiary	Unknown	None	
1584	3.6601	0.7335	20.04	0.273	1.36	Syphilis, tertiary	Unknown	None	
1641	4.6493	1.0217	21.97	0.0107	0.244	Syphilis, tertiary	3 years	None	
1543	6.2927	1.2850	20.42	0.0629	0.259	Syphilis, tertiary	5 years	None	
1663	6.2400	1.6750	26.84	None	None	Tabes	Unknown	None	
1587	8.2021	2.3845	28.86	1.51	5.24	Chancroid	3 weeks	Trace	Dietary
1518	6.2000	1.5708	25.33	None	None	Tertiary syphilis	Denied	Trace	Dietary
1595	4.4510	0.8900	19.99	0.0112	0.1561	Occupational dermatitis	Unknown	Trace	Dietary
1542	5.6875	1.2602	22.15	0.068	0.396	Congenital syphilis	16 years	0.047	Dietary
1520	4.0560	1.0143	22.26	None	None	Lupus vulgaris	6 months	0.051	Dietary or medication
1594	5.6870	1.3312	23.40	0.234	1.001	Tertiary syphilis	Unknown	0.0522	Dietary
1588	6.5015	1.3809	21.23	6.4	30.1	Hydrocele	Unknown	0.0644	Dietary
1579	5.0798	1.1268	22.10	0.590	2.67	Acne cachecticorum	4 years	0.069	Dietary
1283	4.2035	0.9642	22.94	0.012	0.051	Tertiary syphilis	Denied	0.0769	Dietary
1489	5.0406	1.1079	21.97	0.0496	0.225	Pruritis	Unknown	0.087	Dietary or medication
1596	5.4645	1.2449	22.78	0.0548	0.241	Optic atrophy	4 years	0.12	Medication

1544	3.6871	0.7849	21.28	None	None	Syphilis (?)	Denied	0.14	Medication
1541	4.9726	1.0779	21.69	0.0652	0.301	Tertiary syphilis	Denied	0.1005	Medication
1172	5.9067	1.4437	24.44	0.017	0.069	Seborrheic dermatitis	30 years	0.105	Using ungt. sulphur
1578	41.5774	0.9074	23.90	0.132	0.561	Tertiary syphilis	6 years	0.1561	Medication
1173	5.1458	1.0635	20.67	0.00961	0.047	Pruritis	2 weeks	0.16	Medication
1638	6.5644	1.5514	22.79	0.0152	0.0644	Tertiary syphilis	5 years	0.17	Medication
1600	5.9534	1.3016	21.86	0.0168	0.0769	Acne vulgaris	1 year	0.1761	Using lotio alba and R. & S.
1606	5.2574	1.1914	22.66	0.190	0.840	Tertiary syphilis	Denied	0.196	Medication
1589	5.2806	1.2385	23.42	0.141	0.605	Tertiary syphilis	Denied	0.225	Also had herpes zoster, probably arsenical
1645	4.1525	0.9659	23.74	None	None	Acne vulgaris	1½ years	0.241	Using lotio alba and R. & S.
1531	4.0764	0.9718	23.83	0.0122	0.0514	Pruritis	15 years	0.250	Using lotio alba and calamine and zinc lotion
1515	5.0366	1.2070	23.96	0.0697	0.16	Secondary syphilis	6 months	0.259	Medication
1490	5.2200	1.3061	25.05	None	None	Iritis	Unknown	0.259	Medication
1639	5.3136	1.2134	23.96	0.0188	0.0824	Syphilis	Denied	0.301	Medication
1519	5.5749	1.2142	21.77	None	None	Urticaria	9 weeks	0.396	Using lotio alba, calamine and zinc, and R. & S.
1538	4.2039	0.9498	22.59	0.0237	0.105	Rosacea	6 months	0.479	Using lotio alba and R. & S.
1492	5.7400	1.0023	18.506	0.298	1.61	Rosacea	6 years	0.514	Medication
1569	6.3467	1.5012	23.66	0.0473	0.250	Epithelioma	6 months	0.55	Probably medication
1605	5.4635	1.1583	21.10	None	None	Cellulitis	1 month	0.551	Using ungt. ichthyol
1580	4.6539	1.3945	29.96	0.143	0.479	Scabies	3 months	0.605	Using ungt. sulphur
1601	4.4955	1.0244	22.78	None	None	Pityriasis rosea	1 week	0.704	Using lotio alba, calamine and zinc, and R. & S.
1347	5.7105	1.4140	24.76	0.035	0.14	Secondary syphilis	5 months	0.717	Medication
1597	4.8997	1.1462	23.39	0.272	1.16	Congenital syphilis	23 years	0.78	Medication
1517	6.8050	1.5250	22.40	0.044	0.196	Secondary syphilis	2 months	0.824	Medication
1588	5.4953	1.1488	20.90	0.102	0.487	Tertiary syphilis	10 years	0.84	Undoubtedly medication
1607	5.3835	1.2285	22.82	0.162	0.717	Ulcer tongue	2 years	0.873	Medication
1577	3.7895	0.8525	22.52	0.158	0.704	Tertiary syphilis	Unknown	1.001	Undoubtedly medication
1583	6.9990	1.6412	23.46	0.428	1.82	Tertiary syphilis	Unknown	1.16	Undoubtedly medication
1585	4.4425	0.9182	20.66	1.35	6.53	Tertiary syphilis	Unknown	1.36	Undoubtedly medication
1604	5.2316	1.1311	21.62	None	None	Tertiary syphilis	10 years	1.609	Undoubtedly medication
1591	5.0250	1.0563	21.02	None	None	Secondary syphilis	6 months	1.61	Undoubtedly medication
1590	4.1708	0.8440	20.23	None	None	Acne vulgaris	1 year	1.82	Undoubtedly medication
1239	5.7306	1.2488	21.80	0.0175	0.087	Congenital syphilis	25 years	1.92	Undoubtedly medication
1516	6.0065	1.4514	24.16	0.388	1.609	Acne vulgaris	6 months	1.99	Undoubtedly medication
1487	5.7156	0.0574	01.004	None	None	Secondary syphilis	6 months	2.67	Undoubtedly medication
1537	4.5900	1.0611	22.899	Trace	Trace	Herpes zoster	2 weeks	4.41	Undoubtedly medication
1540	6.2463	1.3457	21.22	None	None	Tertiary syphilis	Unknown	5.24	Undoubtedly medication
1644	4.8752	1.0022	21.17	None	None	Tertiary syphilis	5 years	6.53	Undoubtedly medication
1642	6.1827	1.4914	24.22	None	None	Tertiary syphilis	20 years	30.1	Undoubtedly medication

was 0.4929, 0.7742, 0.8051, and 0.7774 mg. metallic arsenic, respectively. The variations are probably due to different degrees of suspension.

Sulphur ointments showed one sample with a trace of arsenic and the other with 0.3141 mg. arsenic per hundred grams. Analyses of lotio alba showed that arsenic in amounts of 0.361 and 0.561 mg. was found in specimens which were being used in the clinic. Ichthyol also contained arsenic to the extent of 0.334 mg. per hundred grams of drug.

SUMMARY

As a result of the examination of the blood, urine, hair and milk from about 200 subjects we can conservatively state that arsenic is found "normally" in a large number of persons, depending on the character of their food, drink, medication and environment. It is absent in persons on a diet restricted in the amount of sweets, vegetables, meat, etc., and it is present in varying amounts in persons applying remedies containing ingredients contaminated with arsenic to inflamed or ulcerating skin surfaces, and to a greater extent in persons receiving arsenic either by mouth or intravenously or connected with the arsenicals in a preparative way.

"Normal" is interpreted as meaning the result found when a large number of persons are examined. Tables 1 to 7 show that it is not necessary for every person to show a positive test at all times. These tables show to some extent that all drugs and foods are contributing factors in the detection of arsenic in human beings, depending, of course, on the method of manufacture and the preparation of the food. Arsenic is also secreted in the milk and is found in hair and skin tissue.

It was difficult to obtain specimens from a large number of normal persons in whom we could control absolutely the character of their diet. In the few who could be relied on the analyses showed quite definitely the absence of the drug where an arsenic-free diet had been carried out. Most of our specimens were from patients with the various common skin diseases such as eczema, urticaria, psoriasis, acne, etc. In some of these patients disordered metabolism is a contributing factor and a possible cause for the variations in the amounts of arsenic found.

Consideration of the results in the tables shows that a complete history of the subject is necessary before arriving at a decision regarding the arsenic content of the urine, blood or even of the organs. In the determination of the amount found "normally" in the body fluids the ideal way would be to hospitalize the subject in order to regulate the nature and amount of food ingested and to check up false statements regarding medication. Consideration of the "normal" arsenic is necessary from a medicolegal point of view. This investigation shows that

it may be a normal constituent of the body under the conditions studied and that excessive amounts should always be considered with suspicion in patients who present themselves for examination and medication.[21]

---

21. The following references also bear on this subject:

Ayres, Jr., S.: Scleroderma as a Possible Manifestation of Chronic Arsenic Poisoning, Arch. Dermat. & Syph. **2**:747 (Dec.) 1920.

Effront, J.: Acquired Tolerance of Beer Yeast for Arsenic; Mechanism of the Acquired Tolerance of Micro-Organisms for Toxic Substances, Reunion soc. belge biol., 1920, p. 99; Physiol Abstr. **5**:437, 1920.

Gautier, A.: Localisation, elimination, et origines de l'arsenic chez les animaux, Compt. rend. **130**:284, 1900. La fonction menstruelle et le rut des animaux. Rôle de l'arsenic dans l'economie, Compt. rend. **131**:361. 1900. L'arsenic existe normalement chez les animaux et se localise surtout dans leurs organes ectodermiques, Compt. rend. **134**:1394, 1902. A propos de la Note de M. G. Bertrand, presente les observations suivantes, Compt. rend. **135**:812, 1902.

Foreign Letters: Hydrogen Arsenic Poisoning in Submarines, J. A. M. A. **73**:1148 (Oct. 11) 1919. Government Control of Antisyphilitic Arsenicals, J. A. M. A. **78**:595 (Feb. 28) 1922.

Kohn-Abrest, E., and Bouligaud: Accidental Presence of Arsenic, in 1916, in Chemical Products and in Some Foods, Ann. chim. anal. **22**:85, 107, 128, 1917.

Niven, J.: Report to the Hospitals Subcommittee of the Manchester Sanitary Committee of the Outbreak of Arsenical Poisoning Now in Progress in Manchester, Lancet **2**:1752, 1900. The Beer Poisoning Epidemic, Lancet **1**:570, 1901.

Shattuck, F. C.: Some Remarks on Arsenical Poisoning with Special Reference to Its Domestic Sources, Med. News **62**:589, 1893.

URICACIDEMIA

BASED ON A STUDY OF 1,500 BLOOD CHEMICAL ANALYSES*

HENRY M. FEINBLATT, M.D.

BROOKLYN

Research in blood chemistry and its clinical application has been almost entirely an American achievement. When it is recalled that this whole subject is now only in its infancy, the widespread use of blood chemical analyses is quite remarkable. Ten years ago accurate clinical methods for the determination of blood urea, uric acid, and creatinin were practically unknown. Closely following the invention of suitable technical procedure, many conclusions have been stated and reiterated as to the significance of high blood concentration of these substances. Some of them are based on slow, painstaking scientific work; others are largely the result of ingenious surmises supported by cases selected to prove the point.

This paper deals with the interpretation of high uric acid figures in the blood. Before 1912 there had been practically no work on this theme. The fact that in gout there was an excess of uric acid in the blood, which could be revealed by Garrod's string test, just about comprised our knowledge at that time.

Folin and Denis,[1] in 1912, opened this whole field when they described a convenient and accurate method for the determination of the uric acid of the blood. Later they observed that in gout, lead poisoning and leukemia, high blood concentration figures are apt to be found. They[2] emphasized the absence of relationship between the amount of uric acid and that of total nonprotein nitrogen (or urea nitrogen) in the blood.

An analysis made by me[3] of twenty-nine cases of uremia showed this lack of parallelism very strikingly. Thus two patients, each with 375 mg. of urea nitrogen per 100 c.c., gave uric acid readings of 35.7 and 5, respectively. In that series, uric acid was invariably increased, but there was an absence of proportion in the retention figures.

---

* From the Clinical Laboratory, Department of Internal Medicine, Long Island College Hospital.

1. Folin, O., and Denis, W.: A New (Colorimetric) Method for the Determination of Uric Acid in Blood, J. Biol. Chem. **13**:469 (Nov. 30) 1912.

2. Folin, O., and Denis, W.: Protein Metabolism from the Standpoint of Blood and Tissue Analysis, J. Biol. Chem. **14**:29, 1913.

3. Feinblatt, H. M.: Uremia: An Analysis of Twenty-Nine Cases, New York M. J., awaiting publication.

Folin and Denis [4] in 1915 stressed the necessity of correlating all uric acid readings with those of the total nonprotein nitrogen (or urea nitrogen). They divided bloods into four classes: (1) those with retention of uric acid alone; (2) those with retention of the total nonprotein nitrogen but not of uric acid; (3) those with retention of both, and (4) those with retention of neither. They pointed out that in gout the uric acid alone is apt to be increased, while in nongouty arthritis both substances are usually retained.

The capriciousness of uric acid was later noted by Pratt [5] in his studies on gout. In sixteen gouty patients he found no relation between the degree of retention of uric acid and the severity of the symptoms; and in two subjects with marked uratic tophi the blood values were within normal limits.

Myers, Fine and Lough [6] were impressed with the value of uric acid retention as an early sign of chronic interstitial nephritis. They reported thirty selected cases with high uric acid figures and no, or only moderate, retention of urea, which appeared to be cases of early chronic interstitial nephritis.

Chace and Myers [7] stated their opinion as follows: "An increase in the uric acid of the blood would appear to be of considerable value as an early diagnostic sign of incipient nephritis." In their tables most of the cases showed concurrent retention of urea, some even of creatinin. They certainly could not be considered as instances of specific retention of uric acid. Furthermore, the use of selected cases instead of routine series greatly limits the value of the work as a scientific contribution.

The questions immediately arise: Granted that uric acid retention may occur in early chronic interstitial nephritis, how uniformly does this happen? Is retention of uric acid peculiar to chronic interstitial nephritis, or, if it does occur in other conditions, can the latter be differentiated readily? These questions must be answered definitely before one can safely interpret uricacidemia as an evidence of nephritis.

Baumann [8] and others, in 1919, reported high uric acid readings in 74 per cent. of 100 cases of early nephritis. The study was largely

4. Folin, O., and Denis, W.: The Diagnostic Value of Uric Acid Determination in Blood, Arch. Int. Med. **16**:33 (July) 1915.

5. Pratt, J. H.: The Uric Acid in Blood in Gout, Am. J. M. Sc. **151**:92 (Jan.) 1916.

6. Myers, V. C.: Fine, M. S., and Lough, W. G.: The Significance of the Uric Acid, Urea and Creatinin of the Blood in Nephritis, Arch. Int. Med. **17**: 570 (April) 1916.

7. Chace, A. F., and Myers, V. C.: The Value of Recent Laboratory Tests in the Diagnosis and Treatment of Nephritis, J. A. M. A. **67**:929 (Sept. 23) 1916.

8. Baumann, L.; Hansmann, G. H.; Davis, A. C., and Stevens, F. A.: The Uric Acid Content of Blood Compared with the Renal Dietary Test, Arch. Int. Med. **24**:70 (July) 1919.

TABLE 1

Name	Age	Sex*	Blood Chemistry				Diagnosis	Outcome	Complications	Evidences of nephritis
			Uric Acid	Urea N.	Creatinin	Sugar				
D. A.	42	♀	12.5	15	1.5	105	Hyperemesis gravidarum	D.	Chronic myocarditis	None
C. K.	42	♂	8.3	20	2	80	Chronic glomerular nephritis	I.		Hypertension, albumin, casts, retinal hemorrhages and optic neuritis; phenolsulphonephthalein, 35 per cent.
A. M.	37	♂	8.2	9	1.3	96	Duodenal ulcer	I.	Secondary anemia	None
J. D.	20	♂	7.13	11.5	1.5	87	Illuminating gas poisoning	D.	Bronchopneumonia	None
P. L.	43	♂	7.13	16.7	1.67	100	Healed duodenal ulcer, with hour-glass contraction	I.	Constipation	None
E. G.	63	♀	6.2	12	1.3	133	Benign hypertension	I.	Obesity	Blood pressure 240/110
M. W.	43	♂	6	9.6	1.5	117.6	Lethargic encephalitis	I.		Blood pressure 180/120; trace of albumin, hyaline and granular casts
			3.8	12	1.75	105				
			4.26	9	1.8	170				
W. R.	18	♂	6	15	1.4	100	Aortic and mitral regurgitation, rheumatic	I.	Hypertrophied tonsils	None
H. W.	25	♀	5.5	12	1.5	96	Lacerations of cervix and perineum	I.	Secondary anemia	None
P. S.	35	♀	5	10	1.3	100	Influenza and bronchopneumonia	R.	Pregnancy	None
L. L.	63	♂	5	12	1.67	90	Adenoma of prostate	R.		None
R. N.	64	♂	5	15	1.4	100	Chronic cholecystitis	I.	Syphilis	None
M. B.	72	♀	5	10	1.5	333	Illuminating gas poisoning	I.		None
E. B.	53	♀	5	13	1.5	83	Lethargic encephalitis	I.		Blood pressure 155/95; few haline casts
M. A.	47	♀	5	12.75	1.35	100	Cerebral thrombosis, syphilitic	D.		None
L. H.	21	♂	4.53	8.6	1.67	87	Traumatic synovitis (knee)	I.		None
P. F.	62	♂	4.53	15	1.36	105	Hyperpituitarism	I.	Arteriosclerosis; diverticulum of urinary bladder	Blood pressure 150/75
C. L.	35	♂	4.53	17.6	1.5	80	Chronic cholecystitis	I.	Hypermotility; myositis	None
W. E.	36	♂	4.53	12	1.5	100	Chronic appendicitis	I.	Periduodenal adhesions	None
B. F.	30	♀	4.53	12.5	1.58	77	Intermittent pyelonephrosis; one kidney absent	R.	Pregnancy	Pyelonephrosis
K. M.	16	♂	4.5	13.6	1.7	75	Cellulitis of arm (Streptococcus hemolyticus)	R.		None
M. H.	32	♂	4.5	10	1.5	109	Oral sepsis	R.	Constipation	None

L. G.	40	♂	4.4	16.6	1.5	111	Neurasthenia	U.		None
T. E.	60	♂	4.2	17.6	2	110	Hypertrophied prostate	I.	Retention of urine	None
A. C.	49	♂	4.2	13.4	1.3	109	Cerebral hemorrhage	I.	Secondary anemia; possible chronic glomerular nephritis	Hypertension; casts; albumin
W. H.	51	♂	4.2	17	1.6	125	Benign hypertension	I.	Aortic stenosis	Blood pressure 160/140; albumin; specific gravity 1.012-1.022
M. B.	50	♀	4.2	12	1.4	200	Lobar pneumonia	R.	Mitral regurgitation	Blood pressure 160/80; casts; trace of albumin
J. A.	44	♀	4.2	15	1.5	80	Multiple arthritis	I.	Hypertrophied tonsils	Blood pressure 165/105
S. C.	25	♂	4.15	13.6	1.5	100	Bell's palsy	I.		None
M. S.	46	♀	4.15	10	1.67	111	Benign hypertension; arteriosclerosis	I.	Chronic colitis; possible early nephritis	Blood pressure 190/120; hyaline casts; phenolsulphonephthalein, 40 per cent.
V. C.	64	♀	4.15	10	1.5	181	Arteriosclerotic kidney	I.	Fibrositis, knee; possible early nephritis	Blood pressure 218/85; phenolsulphonephthalein, 50 per cent.; specific gravity, 1.011-1.020; night urine, 740 c.c.; day urine, 735 c.c.; hyaline and granular casts
A. S.	33	♂	4.15	17.6	1.43	90	Tertian malaria	R.	Tertiary syphilis	None
J. K.	41	♂	4.1	15	1.8	143	Chronic cholecystitis	I.	Secondary anemia	None
L. K.	32	♂	4.1	12	1.3	111	Hemorrhoids	I.	Secondary anemia	None
H. H.	56	♂	4.1	15	1.5	133	Cerebral concussion	R.		None
P. T.	41	♂	4.1	12	1.5	125	Pott's disease	I.	Pulmonary tuberculosis; pleurisy	None
T. S.	49	♀	3.83	15	1.36	100	Benign hypertension	I.	Menopause	Blood pressure 190/110; phenolsulphonephthalein, 57 per cent.; specific gravity, 1.009-1.025; night urine, 220 c.c.; day urine, 1.030 c.c
R. T.	18	♂	3.83	15	1.5	105	Acute bronchitis	R.		None
O. M.	39	♂	3.83	8.3	1.5	333	Typhoid fever	R.		Albumin; few granular casts
M. T.	26	♂	3.8	20	1.5	125	Acute follicular tonsillitis	R.		None
E. V.	18	♀	3.8	19	1.3	100	Idiopathic epilepsy	I.		None
F. L.	20	♀	3.8	9	1.4	125	Chronic cholecystitis	I.		None
R. K.	30	♂	3.6	15	1.6	100	Cerebral thrombosis	I.	Tonsillitis; otitis media	None
E. V.	45	♀	3.55	13.6	1.5	181	Essential hematuria	I.		Albumin; hematuria; phenolsulphonephthalein, 60 per cent.; specific gravity, 1.012-1.016
F. V.	31	♀	3.5	15	1.6	133	Urticaria	I.		None
J. J.	27	♀	3.5	12	2	111	Chronic glomerular nephritis	I.		Blood pressure 245/165; albumin; specific gravity, 1.010-1.018
C. S.	60	♀	3.5	16.7	1.5	333	Hypernephroma, right kidney	R.		Blood pressure 160/80; trace of albumin

* In this column, ♂ indicates male, and ♀ female.

limited to instances of nephritis and arteriosclerosis. High urea figures were frequently associated.

The obstetrician has utilized uric acid determinations for diagnostic purposes. Williams,[9] in 1921, demonstrated that in eclampsia, pre-eclamptic toxemia, and toxic hyperemesis gravidarum, retention of only uric acid is the rule. With delivery and recovery from the symptoms the blood value returns to normal.

Chauffard [10] attributes a common origin to gout and nephrolithiasis. He found uric acid retention to be the rule in both conditions. In three dogs he obtained much higher uric acid readings from the portal vein than from the peripheral veins. He ascribes uricadidemia principally to a loss of the uricolytic function of the liver.

Perroncito [11] likewise showed that after removal of the liver the blood uric acid rapidly rose while the urea values fell abruptly. These observations would suggest that uric acid is not very readily eliminated even by the undamaged kidney and that high blood concentration often results from diminution of the uricolytic function of the liver.

TECHNIC

The blood was drawn before breakfast on the day following that of admission. These patients were not kept on a purin free diet. At one time it was considered essential to make the determination only after the patient had been subjected to a purin free diet for a period of two weeks. Denis'[12] studies showed that this is not necessary. She observed that in normal men there is no increase in the blood uric acid values even after the ingestion of large amounts of purins.

The technic employed was that of Folin.[13]

MATERIAL STUDIED

Out of 1,500 blood chemical analyses taken in the wards of the Long Island College Hospital, there were separated all of those cases, regardless of diagnosis, which conformed to the following specifications: Uric acid, 3.5 mg. per 100 c.c. or more; urea nitrogen, 20 mg. per 100 c.c. or less; creatinin, 2 mg. per 100 c.c. or less. Blood chemical analyses were made in medical cases as a matter of routine. This scheme of mechanical winnowing must obviously yield results which are entirely

9. Williams, J. L.: Increased Amount of Uric Acid in Blood in Toxemias of Pregnancy, J. A. M. A. **76**:1297 (May 7) 1921.

10. Chauffard, A.: Brodin, P., and Grigaut, A.: L'Hyperuriceme dans la Goutte et dans la Gravelle, Presse méd. **28**:905 (Dec. 15) 1920.

11. Perroncito, A.: Extirpation of liver, Riforma med. **36**:830 (Sept. 11) 1920.

12. Denis, W.: Effect of Ingested Purins on Uric Acid Content of Blood, J. Biol. Chem. **23**:147 (Nov.) 1915.

13. Folin, O.: A Laboratory Manual of Biological Chemistry, 1919, p. 193.

free from prejudices. Because of the large number of cases considered, the findings may be considered as fairly representative, subject, of course, to any special types of patients treated at this institution.[14] There was no selection of cases. This system of mechanical winnowing was utilized in the study of creatininemia [15] which condition, thus investigated, proved to be associated with grave renal insufficiency in 100 per cent. of instances.

The data on all of the uricacidemic patients are tabulated in Table 1.

Out of 1,500 routine analyses, forty-seven patients exhibited uricacidemia without concurrent retention of urea or creatinin. From a glance at Table 1 it becomes apparent that this finding may be present in many and varied conditions.

Table 2 summarizes the diagnosis in these forty-seven cases.

TABLE 2.—SUMMARY OF CLINICAL DIAGNOSES IN FORTY-SEVEN URICACIDEMIC SUBJECTS

Diagnosis	No. Cases
Benign hypertension	5
Chronic cholecystitis	4
Duodenal ulcer	2
Chronic glomerular nephritis	2
Illuminating gas poisoning	2
Lethargic encephalitis	2
Cerebral thrombosis	2
Miscellaneous conditions (occurring only once)	28

Because of the often expressed view that uric acid retention is to be considered as an evidence of nephritis, this series of cases was studied with especial care as to other signs of renal lesions. The urine, the renal test meal, the phenolsulphonephthalein test, the blood pressure and the eyegrounds were among the other avenues of approach. Thus studied, two of the cases were diagnosed as chronic glomerular nephritis, five as benign hypertension (probably associated with arteriosclerosis of the vasa afferentia to the renal glomeruli), and one as pyelonephrosis. The remaining thirty-nine cases were not considered as belonging to the kidney group.

Altogether, thirty of the forty-seven patients revealed none of the signs of renal disease, although these were systematically looked for. In seventeen cases there were present some of the evidences pointing to the kidney, but in nine of these the evidence was considered insufficient to justify the diagnosis of a nephropathy.

The multiplicity of conditions in which uricacidemia may eventuate should indicate that this finding must be interpreted with considerable

14. The cases treated at the Long Island College Hospital are fairly representative in type.

15. Feinblatt, H. M.: Creatininemia Based upon a Study of 1,500 Blood Chemical Analyses, Am. J. M. Sc., awaiting publication.

caution. To base a diagnosis of chronic interstitial nephritis merely on uric acid retention appears to be altogether unwarranted.

In a recent Harvey lecture, Folin [16] took a very conservative stand on the subject of uricacidemia. He said:

> A very important point yet to be solved is the question whether the kidneys are or are not selective with reference to the excretion of the nitrogenous waste products. Are the kidneys of those who have or are going to have gout damaged specifically with reference to their power of eliminating uric acid? That is too fundamental a problem to permit any ill founded dogmatism. It certainly is true that in those who have gout one is likely to find abnormally high uric acid accompanied by a perfectly normal level for all the other nitrogenous constituents. Whereas, in nephritis we may have very high levels of nitrogen retention and substantially normal uric acid values. I hesitate to say anything very definite on this whole question.

Two facts invalidate uric acid as an index to renal efficiency: viz., the frequent occurrence of retention in totally unrelated conditions and the often observed lack of parallelism with urea and creatinin in known cases of nephritis.

It is by no means rare in uremia, while the patient is comatose and the creatinin and urea nitrogen figures are rising, to note a marked drop in the blood uric acid figure. Three such instances are described below in Table 3.

TABLE 3.—SUDDEN DROP IN URIC ACID CONCENTRATION IN THREE FATAL CASES OF UREMIA

Name	Age	Sex§	Date of Analysis	Uric Acid	Urea N.	Creatinin	Sugar	Date of Death
V. H.*	25	♂	2/17/20	6.2	50	7.5	133.3	2/28/20
			2/21/20	12.5	300	8.6	200	
			2/24/20	8.2	300	15	143	
			2/27/20	5	375	20	138	
H. G.†	43	♀	3/26/21	8.85	80	3.75	166	4/ 5/21
			3/29/21	4.15	60	4.28	111	
H. A.‡	29	♀	2/17/19	10	100	6.5	142	2/21/19
			2/19/19	4.3	115	6.6	200	

* Acute exacerbation of chronic glomerular nephritis. Patient was uremic and died in coma.

† Acute exacerbation of chronic glomerular nephritis, confirmed by necropsy.

‡ Coagulation necrosis of renal tubules due to mercuric chlorid poisoning, complicated by gangrenous stomatitis and gastro-enteritis. Confirmed by necropsy.

§ In this column, ♂ signifies male, ♀ female.

In uremia a certain rough ratio can be detected between the urea nitrogen and the creatinin figures. They rise together as death approaches and descend proportionately with improvement. No such parallel exists in the case of uric acid. The data recorded in Table 3 are irreconcilable with the view that uricacidemia is a sensitive test of slight degrees of renal inefficiency.

16. Folin, O.: Blood Analysis and Its Applications: Harvey Society Lectures, Delivered Feb. 28, 1920, p. 109.

It is not my purpose to attempt to annihilate all interpretations placed on high blood uric acid concentration. The common finding of uricacidemia, without retention of total nonprotein nitrogen, in gout, nephrolithiasis, leukemia, lead poisoning, hyperemesis gravidarum and eclampsia, has been abundantly shown. However, it should be stressed that this state may also obtain in a vast number of unrelated conditions, under circumstances which are not yet understood. The blood uric acid, therefore, cannot be considered in any sense as diagnostic but should be thought of as a symptom. Given an arthritis of the small joints, uricacidemia furnishes an additional sign in favor of gout; or, in a case of hyperemesis gravidarum a high uric acid reading indicates that the condition is toxic rather than neurotic. Any effort to assign to uricacidemia a uniform interpretation, i. e., chronic interstitial nephritis, is an assumption not warranted by known facts.

CONCLUSIONS

1. Uricacidemia may occur in a large number of totally unrelated conditions.

2. A study of 1,500 persons with routine blood chemical analyses showed that the great majority of patients with high blood concentration of uric acid but not of urea nitrogen or creatinin exhibited no evidence at all of early chronic interstitial nephritis.

3. In uremia there is a lack of parallelism between the figures for uric acid, on the one hand, and those for urea nitrogen and creatinin on the other. Frequently, with mounting urea nitrogen and creatinin, and deepening coma, terminating in death, the concentration of uric acid in the blood takes a marked drop. There is often but slight retention of uric acid in moribund uremics.

4. The blood uric acid reading is of no value unless correlated with that of urea nitrogen (or total nonprotein nitrogen). A high concentration may be interpreted as a symptom, along with the other manifestations, but is in no sense diagnostic in itself of early chronic interstitial nephritis or any other condition.

ACCURATE CRITERIA FOR DIFFERENTIATING ANEMIAS *

RUSSELL L. HADEN, M.A., M.D.

KANSAS CITY, KAN.

In differentiating types of anemia, clinicians are constantly making use of the relationship between the number of erythrocytes and the hemoglobin. This relationship is usually expressed as the color index. In view of its wide use, it is surprising how vague are the ideas of the factors on which the color index is fundamentally based. This is due, in some measure, to the common use of only relative methods for the determination of hemoglobin. To arrive at precise information, it is necessary to have the hemoglobin content expressed in exact terms of grams per hundred cubic centimeters. Suggestions for a normal hemoglobin standard on this basis have been pointed out elsewhere.[1]

Very few careful studies of color index have been made. It is not even universally agreed that the color index of normal blood is always 1.00. Meyer and Butterfield,[2] on the basis of a very few observations with hemoglobin determinations by the spectroscopic method, conclude that such is always true.

A proper understanding of the relationship between hemoglobin and erythrocytes presupposes a knowledge of the number, the size and the hemoglobin content of the red cells. It is commonly thought that color index is an expression indicating the percentage of hemoglobin in the cells. This is true only when the cells are of normal volume. The index in reality depends on both the size and the hemoglobin content. As ordinarily determined, the size of the cell is not taken into consideration.

In 1903 Capps [3] emphasized the value of the study of the size of the erythrocyte. He introduced the term "volume index" to express the volume of the red cell as compared with the normal. Since that time little use has been made of the index. Only two articles concerning it have appeared in the literature. Capps determined the volume of the red cells with Daland's hematocrit, which is relatively crude and gives no information on which the exact volume of the average cell may be calculated. His hemoglobin determination was made by a relative

* From the Department of Medicine, University of Kansas, School of Medicine.

1. Haden, R. L.: J. A. M. A. **79**:1496 (Oct. 28) 1922.
2. Meyer, Erick and Butterfield, E. E.: Arch. Int. Med. **14**:94 (July) 1914.
3. Capps, J. A.: J. M. Research **5**:367, 1903.

method, namely, that of von Fleischl, the results of which cannot be translated into grams or be made the basis for calculating the hemoglobin content of the average red cell.

Capps emphasized that in pernicious anemia the volume of the average cell is greater than normal, and that in secondary anemia it is smaller. In pernicious anemia he found the color index greater than the volume index. The importance of this will be pointed out later. He did find a volume index greater than 1.00 in conditions other than pernicious anemia, however. He did not calculate in any instance the actual volume and hemoglobin content of the average cell. Wroth [4] reports a few cases which confirm Capps conclusions. Larrabee,[5] working with a slightly different technic, did not have such constant results.

It is certainly true that few clinicians ever make use of the volume index. In some of the most recent discussions of anemia no mention is even made of its value and use in differential diagnosis in certain types of anemia.

In connection with a study of blood volume, Hooper, Smith, Belt and Whipple [6] have recently suggested a method for the accurate determination of the volume of the mass of red cells. A relatively large quantity of blood (10 c.c.) is taken, and an anticoagulant isotonic with the blood is used, thus preventing any shrinking or swelling of the cells. Much more constant and exact results are obtained than is possible with either the hematocrit of Daland or by adding an indefinite quantity of solid potassium oxalate directly to the blood. From the data obtained, together with the counts, the exact volume of the average cell may be calculated. Likewise there is now available for hemoglobin estimation the very exact oxygen capacity method of Haldane [7] as adapted by Van Slyke [8] to his blood gas apparatus. The results are expressed in grams per hundred cubic centimeters.

I have employed these two precise methods in a study of the blood of a large number of normal persons and in anemia of different types. The volume and color index have been calculated for each blood examined. Since the color index does not express the amount of hemoglobin per unit volume of cell, I have calculated a new index which does express this value. This index I have called the "saturation index" to indicate that it shows the relative amount of hemoglobin in the red cells as compared with the normal. Only in normal blood is it

4. Wroth, P.: Bull. Johns Hopkins Hosp. **18**:59, 1907.
5. Larrabee, R. C.: J. M. Research **24**:15, 1911.
6. Hooper, Smith, Belt and Whipple: Am. J. Physiol. **51**:205, 1920.
7. Haldane, J. S.: J. Physiol. **22**:298, 1898.
8. Van Slyke, D. D.: J. Biol. Chem. **33**:31, 1918.

synonomous with the color index. The saturation index shows the same relation of the hemoglobin to the cell as the volume index does of the volume. The color index depends on these two indices.

From the data obtained on the absolute volume of the red cells and the hemoglobin I have calculated the exact volume and the exact hemoglobin content of the average red cell in each blood examined, and in turn the exact percentage of hemoglobin in the red cells.

METHODS

All determinations have been made on venous blood. The following procedure has been followed: 20 c.c. of blood is withdrawn by venipuncture, 10 c.c. of which is immediately run into a 15 c.c. graduated centrifuge tube containing 2 c.c. of 1.6 per cent. sodium oxalate solution and mixed by inversion. The tube is then centrifuged for thirty minutes at 2,500 revolutions per minute and the volume of packed red cells read off. The remaining blood is run into a test tube or flask containing 40 mg. of potassium oxalate and mixed thoroughly. From this oxalated specimen two red cell pipets are filled in the usual manner for counting. The tube or flask is then rotated for five minutes in such a manner as to keep the blood in a thin layer on the sides of the container. The hemoglobin is thus completely converted into oxyhemoglobin. Duplicate hemoglobin determinations are made with the Van Slyke apparatus. The determinations have been made by both the original technic [8] and by the revised method.[9] Identical results have been obtained with the two methods. Van Slyke has shown that the calculation as originally given is incorrect since sufficient allowance was not made for the nitrogen content of the blood. I have made all calculations by the revised method.

In determining the volume of the cells, two centrifuges have been used at different times. The more powerful centrifuge gave slightly better packing (46 per cent. of cells per 5 million cells). All volume results have been figured on this basis. It should be emphasized here that in making volume index determinations, one should determine for the centrifuge used, the volume of cells obtained on centrifuging a blood containing 5 million cells of normal size and consider this as 100 per cent.

In counting the red cells two preparations have been made from each of the two pipets filled and counted. This figure given for the red count thus represents four counts on each blood. Unless satisfactory checks were obtained, the counts were repeated. This is very important since we have found that the most frequent source of error in calculating the indices is in the red counts.

9. Van Slyke, D. D.: J. Biol. Chem. **49**:1, 1921.

The counting chamber and pipets have been certified as correct by the Bureau of Standards. All other pipets used, the hematocrit tubes and the Van Slyke apparatus, have been recalibrated carefully by weighing with water.

The volume of packed cells taken as normal, or 100 per cent., namely, 4.6 c.c. of packed cells for 10 c.c. of blood containing 5 million cells per c.mm., was calculated from the average of many normal bloods. In figuring the color index, 15.6 gm. hemoglobin per hundred cubic centimeters of blood is taken as 100 per cent.[1] The saturation index is calculated by dividing the percentage of hemoglobin by the volume per cent. of packed cells. The calculation of the three indices and the actual volume of hemoglobin content of the average red cell is best illustrated by an example.

Example.—On centrifuging 10 c.c. of normal blood with a red cell count of 5 million per c.mm., 4.6 c.c. of packed cells are obtained. On centrifuging a similar volume of blood from a patient with anemia, 3.1 c.c. are obtained. The red cell count is 2,500,000 per c.mm. The volume index is

$$\frac{\dfrac{\text{Volume R. B. C. obtained}}{\text{Normal volume}}}{\dfrac{\text{R. B. C. per c.mm.}}{5{,}000{,}000}} = \frac{\dfrac{3.1}{4.6}}{\dfrac{2{,}500{,}000}{5{,}000{,}000}} = \frac{0.675}{0.500} = 1.35$$

The hemoglobin content of the blood of this patient is 9.36 gm. per hundred cubic centimeters, or 60 per cent. (Normal Standard). The color index, calculated in the usual way, is 1.20.

The saturation index is $\dfrac{\text{Per cent. of hemoglobin}}{\text{Volume per cent. of cells}} = \dfrac{60}{67.5} = 0.89$

The individual cell volume is $\dfrac{3.1}{2{,}500{,}000 \times 1000 \times 10} = 12.4 \times 10^{-11}$ c.c.

The hemoglobin content of the average cell is

$$\frac{9.6}{2{,}500{,}000 \times 1000 \times 100} = 3.74 \times 10^{-11} \text{ gm.}$$

The actual hemoglobin content of the red cell is $\dfrac{3.74 \times 10^{-11}}{12.4 \times 10^{-11}} = 30.2$ per cent.

Summary.—The blood of this patient, then, has a volume index of 1.35, a color index of 1.20, a saturation index of 0.89, average cell volume of 12.4×10^{-11} c.c., an average cell hemoglobin content of 3.84×10^{-11}, and the hemoglobin in the cells is 30.2 per cent.

METHODS

The findings in normal blood are shown in Tables 1, 2, 3 and 4. It will be noted that the hemoglobin in grams per hundred cubic centimeter per 5 million cells is remarkably constant. The average in the series of normal men, ages 18 to 30, is 15.57 gm. per hundred cubic centimeter, normal ages from 30 to 50, 15.65 gm., and normal women all ages, 15.65 gm. The average of all men and women is 15.6 gm.

TABLE 1.—Normal Men—Ages 18 to 30

Age	R. B. C. in Millions per C.Mm.	Oxygen Capacity per 100 C.c.	Gm. Hb. per 100 C.c.	Hb. in per Cent.	Oxygen Capacity per 100 C.c. per 5 Million Cells	Hemoglobin in Gm. per 100 C.c. per 5 Million Cells	Hematocrit Reading	Volume per Cent. R. B. C.	Volume Index	Color Index	Saturation Index	Volume Average Cell in C.c. × 10^{-11}	Gm. Hb. in Average cell × 10^{-11}	Actual Percentage Hb. in cell
18	4.98	21.70	16.20	103	21.98	16.40	45.0	98	0.99	1.04	1.05	9.1	3.28	36.0
18	5.10	21.70	16.20	103	21.15	15.85	48.0	104	1.02	1.01	0.99	9.4	3.17	33.6
19	5.20	21.07	15.75	101	20.30	15.15	45.0	98	0.95	0.97	1.02	8.7	3.03	34.8
23	4.80	21.57	16.08	103	22.40	16.70	45.5	99	1.03	1.05	1.02	9.5	3.34	34.6
23	5.62	22.12	16.61	106	19.76	14.75	51.0	110	0.98	0.94	0.96	9.0	2.95	32.6
24	4.87	20.15	15.13	97	20.85	15.55	45.5	99	1.02	0.99	0.97	9.4	3.11	32.9
25	4.84	20.30	15.00	96	20.80	15.50	42.0	91	0.96	0.99	1.04	8.7	3.10	35.1
25	5.26	22.00	16.40	105	20.95	15.65	49.0	106	1.01	1.00	0.99	9.2	3.13	33.6
25	5.49	22.40	16.70	107	20.19	15.20	49.5	107	0.97	0.97	1.00	8.9	3.04	33.9
25	5.50	19.25	16.05	103	19.53	14.60	50.0	108	0.99	0.93	0.95	9.1	2.92	32.2
26	5.36	21.45	16.10	103	20.09	15.05	47.0	102	0.95	0.96	1.01	8.7	3.10	34.2
26	4.98	21.93	16.32	104	21.98	16.40	47.0	102	1.02	1.04	1.02	9.4	3.28	34.8
26	5.00	21.98	16.40	105	21.95	16.40	45.0	98	0.98	1.05	1.07	9.0	3.28	36.2
27	4.70	19.73	14.72	95	21.06	15.70	46.0	100	1.06	1.01	0.95	9.7	3.14	32.2
28	5.57	23.00	17.17	110	20.55	15.35	51.0	111	1.00	0.99	0.99	9.2	3.07	33.6
28	5.09	20.80	15.53	100	20.50	15.30	45.0	98	0.96	0.98	1.02	8.8	3.06	34.6
28	4.72	19.68	14.70	94	20.90	15.60	44.0	96	1.02	1.00	0.98	9.4	3.12	33.2
29	5.09	21.18	15.80	101	20.85	15.55	46.0	100	0.98	0.99	1.01	9.0	3.11	34.2
29	4.52	18.80	14.02	90	20.80	15.50	44.0	96	0.95	0.99	1.04	8.7	3.10	35.1
29	4.99	21.00	15.70	101	20.90	15.60	46.0	100	1.00	1.01	1.01	9.2	3.12	34.2
Average	5.08	21.20	15.82	101	20.85	15.57	46.5	101	1.00	1.00	1.00	9.2	3.11	33.9

TABLE 2.—Normal Men—Ages 30 to 50

Age	R. B. C. in Millions per C.Mm.	Oxygen Capacity per 100 C.c.	Gm. Hb. per 100 C.c.	Hb. in per Cent.	Oxygen Capacity per 100 C.c. per 5 Million Cells	Hemoglobin in Gm. per 100 C.c. per 5 Million Cells	Hematocrit Reading	Volume per Cent. R. B. C.	Volume Index	Color Index	Saturation Index	Volume Average Cell in C.c. × 10^{-11}	Gm. Hb. in Average cell × 10^{-11}	Actual Percentage Hb. in cell
30	4.80	20.20	15.10	97	21.10	15.70	46.5	99	1.03	1.01	0.98	9.5	3.14	33.2
30	4.73	18.45	13.80	89	19.60	14.60	44.0	95	1.00	0.94	0.94	9.2	2.92	31.9
31	4.40	19.35	14.41	93	21.95	16.35	43.0	93	1.05	1.05	1.00	9.7	3.27	33.9
32	5.20	21.57	16.10	103	20.80	15.50	45.0	98	0.95	0.99	1.05	8.7	3.10	35.6
32	5.02	20.80	15.52	100	20.80	15.50	46.0	100	1.00	1.00	1.00	9.2	3.10	33.9
32	4.80	20.92	15.11	96	21.12	15.75	46.0	100	1.00	1.00	1.00	9.2	3.15	33.9
33	4.58	19.43	14.50	93	21.20	15.80	45.0	98	1.06	1.01	0.96	9.8	3.16	32.6
35	5.05	20.72	15.45	99	20.55	15.30	46.0	100	0.99	0.98	1.01	9.1	3.06	34.2
35	5.15	22.13	16.50	106	21.45	16.00	48.0	104	1.01	1.02	1.01	9.3	3.20	34.2
35	5.46	21.65	16.15	104	19.85	14.80	49.0	107	0.98	0.96	0.98	9.0	2.96	33.2
35	4.97	20.65	15.40	99	20.80	15.50	45.0	98	0.99	1.00	1.01	9.1	3.10	34.2
36	4.80	20.65	15.40	98	21.52	16.05	45.0	98	1.02	1.01	0.99	9.4	3.21	33.7
38	4.27	18.75	14.00	89	22.00	16.40	39.5	86	1.01	1.05	1.04	9.3	3.28	35.2
39	5.16	21.50	16.10	103	20.85	15.55	47.5	103	0.99	1.00	1.01	9.1	3.11	34.2
40	4.64	19.20	14.30	92	20.70	15.45	42.0	92	1.00	1.00	1.00	9.2	3.10	33.9
44	5.20	22.65	16.90	108	21.82	16.25	49.0	107	1.02	1.03	1.01	9.4	3.25	34.2
44	4.56	19.00	14.20	91	20.85	15.55	42.0	92	1.01	1.00	0.99	9.3	3.11	33.6
49	4.32	18.75	14.00	89	21.80	16.30	40.0	87	1.00	1.03	1.03	9.2	3.24	34.9
50	4.89	20.90	15.60	100	21.30	15.90	44.5	97	0.99	1.02	1.02	9.1	3.18	34.6
50	5.28	21.55	16.10	103	20.25	15.25	48.0	104	0.99	0.98	0.99	9.1	3.05	33.6
Average	4.865	20.25	15.23	98	21.00	15.65	45.0	98	1.00	1.00	1.00	9.2	3.13	33.9

which figure is taken as 100 per cent. in calculating the hemoglobin in per cent. and in determining the color index and the saturation index. The color and volume index vary only within the limits of error of the determination, namely 0.95 and 1.05. The average is exactly 1.00. The saturation index varies within the same limits with an average of 1.00. The volume and hemoglobin content of the normal cell varies

TABLE 3.—NORMAL WOMEN

Age	R. B. C. in Millions per C.Mm.	Oxygen Capacity per 100 C.c.	Gm. Hb. per 100 C.c.	Hb. in per Cent.	Oxygen Capacity per 100 C.c. per 5 Million Cells	Hemoglobin in Gm. per 100 C.c. per 5 Million Cells	Hematocrit Reading	Volume per Cent. R. B. C.	Volume Index	Color Index	Saturation Index	Volume Average Cell in C.c. × 10^{-11}	Gm. Hb. in Average cell × 10^{-11}	Actual Percentage Hb. in cell
20	4.15	17.70	13.20	84	21.30	15.90	38.0	83	1.00	1.01	1.01	9.2	3.18	34.2
21	3.89	16.23	12.12	78	21.90	15.60	36.0	79	1.01	1.01	1.00	9.3	3.12	33.9
22	4.20	17.49	13.10	84	20.90	15.60	38.0	83	0.99	1.00	0.99	9.1	3.12	33.6
23	4.40	18.07	13.50	87	20.55	15.35	40.5	88	1.00	1.00	1.00	9.2	3.07	33.9
25	4.45	18.60	13.88	88	20.90	15.60	41.0	90	1.01	0.99	0.98	9.3	3.12	33.2
26	4.38	19.90	14.80	95	22.60	16.90	43.0	93	1.05	1.08	1.08	9.7	3.38	34.9
27	4.72	19.56	14.60	94	20.70	15.45	43.0	94	1.00	1.00	1.00	9.2	3.09	33.9
27	3.97	15.90	11.90	76	20.10	15.00	37.0	80	1.02	0.97	0.96	9.4	3.00	32.2
30	4.56	18.75	14.00	90	20.58	15.35	41.0	90	0.99	0.99	1.00	9.1	3.07	33.9
38	3.95	16 20	12.10	78	20.58	15.30	37.0	80	1.01	0.99	0.98	9.3	3.06	33.2
40	4.31	17.98	13 40	86	20.90	15.60	40.0	87	1.02	1.00	0.98	9.4	3.12	33.2
40	4.26	18.05	13.50	86	21.15	15.85	39.0	85	1.00	1.01	1.01	9.2	3.17	34.2
Average	4.26	17.33	13.34	85	21.00	15.65	39.0	85	1.00	1.00	1.00	9.2	3.13	33.9

TABLE 4.—MEN AND WOMEN OF ALL AGES

R. B. C. in Millions per C.Mm.	Oxygen Capacity per 100 C.c.	Gm. Hb. per 100 C.c.	Hb. in per Cent.	Oxygen Capacity per 100 C.c. per 5 Million Cells	Hemoglobin in Gm. per 100 C.c. per 5 Million Cells	Hematocrit Reading	Volume per Cent. R. B. C.	Volume Index	Color Index	Saturation Index	Volume Average Cell in C.c. × 10^{-11}	Gm. Hb. in Average cell × 10^{-11}	Actual Percentage Hb. in cell	
5.08	21.20	15.83	101	20.85	15.57	46.5	101	1.00	1.00	1.00	9.2	3.11	33.9	Average men 18-30
4.865	20.25	15.23	98	21.00	15.65	45.0	98	1.00	1.00	1.00	9.2	3.13	33.9	Average men 30-50
4.26	17.33	13.34	85	21.00	15.65	39.0	85	1.00	1.00	1.00	9.2	3.13	33.9	Average women all ages
4.74	19.86	14.80	95	20.90	15.62	43.5	95	1.00	1.00	1.00	9.2	3.12	33.9	Average all men and women

little. The average volume of the cells in men and women is 9.2×10^{-11} c.c. Starling [10] states that the normal cell volume is 7.22×10^{-11} c.c. Luciani [11] gives it as 6.67×10^{-11} c.c. The former results are based on determinations with models of high magnification. Our figure is higher than the figures quoted. It is possible that some plasma may

10. Starling, E. H.: Principles of Human Physiology, Philadelphia, Lea & Febiger, 1915, p. 818.

11. Luciani, L.: Human Physiology, New York, Macmillan & Co. **1**:105, 1911.

remain in the packed cells giving readings which are somewhat too high. The centrifuge used was a very powerful one, however, and maximum packing was attained so it is difficult to see how any fluid could remain in the interstices between the cells. The average hemoglobin content of the cells varies little in men and women, the average being 3.12×10^{-11} gm. The actual percentage of hemoglobin in the red cells is 33.9. There are no variations from this beyond the limits of error.

Pernicious Anemia.—The findings in pernicious anemia present a marked contrast to those of normal blood (Table 5). The bloods

TABLE 5.—PERNICIOUS ANEMIA

R. B. C. in Millions per C.Mm.	Oxygen Capacity per 100 C.c.	Gm. Hb. per 100 C.c.	Hb. in per Cent.	Oxygen Capacity per 100 C.c. per 5 Million Cells	Hemoglobin in Gm. per 100 C.c. per 5 Million Cells	Hematocrit Reading	Volume per Cent. R. B. C.	Volume Index	Color Index	Saturation Index	Volume Average Cell in C.c. × 10^{-11}	Gm. Hb. in Average cell × 10^{-11}	Actual Percentage Hb. in cell	Remarks
0.74	3.83	2.76	18	25.95	19.35	10.0	21	1.41	1.21	0.86	13.0	2.87	29.2	
1.24	3.53	2.63	17	14.15	10.55	15.0	33	1.31	0.69	0.52	12.1	2.11	17.6	Large spleen
1.38	6.24	4.65	30	22.60	16.85	15.0	33	1.21	1.09	0.90	11.1	3.37	30.5	
1.42	8.90	6.65	43	31.40	23.40	19.5	42	1.47	1.51	1.02	13.5	4.68	34.6	
1.58	9.15	6.80	44	28.85	21.50	21.0	46	1.45	1.39	0.96	13.4	4.30	32.6	
1.64	9.55	7.13	46	29.15	21.75	21.0	46	1.39	1.39	1.00	12.8	4.35	33.9	
1.61	7.79	5.60	37	23.30	17.40	18.0	39	1.22	1.16	0.96	11.2	3.48	31.5	
1.68	8.30	6.20	40	24.75	18.45	18.5	40	1.20	1.20	1.00	11.1	3.69	33.9	Primary aplastic type
1.73	7.90	5.89	38	22.80	17.00	22.0	48	1.38	1.10	0.79	12.7	3.40	26.8	
1.75	9.87	7.55	47	28.20	21.00	21.0	46	1.31	1.31	1.00	12.1	4.20	33.9	Ataxia; fixed pupils
1.81	8.27	6.15	39	22.80	17.00	22.0	48	1.33	1.08	0.82	12.3	3.40	27.8	
1.88	12.33	9.22	59	32.85	24.50	29.0	63	1.68	1.57	0.93	15.5	4.90	31.5	
1.99	10.90	8.30	52	27.60	20.60	26.0	56	1.40	1.30	0.93	12.9	4.12	31.5	
2.10	11.50	8.59	55	27.40	20.45	25.0	54	1.35	1.30	0.96	12.4	4.09	32.6	
2.27	7.98	5.96	38	17.50	13.05	37.0	81	1.77	0.84	0.47	16.3	2.61	16.0	Very marked cord changes
2.33	12.17	9.08	58	26.15	19.50	28.5	62	1.33	1.24	0.94	12.2	3.90	31.9	
2.60	10.18	7.60	49	19.60	14.60	28.0	61	1.17	0.94	0.80	10.8	2.92	27.1	
2.79	13.60	10.20	65	24.50	18.30	32.0	69	1.23	1.16	0.94	11.3	3.66	31.9	
2.98	17.29	12.90	83	29.50	22.00	36.5	79	1.35	1.39	1.03	12.4	4.40	34.9	Remission for six years
3.33	16.40	12.20	80	24.60	18.35	37.0	81	1.20	1.20	1.00	11.1	3.67	33.9	Remission for ten years
1.94	9.75	7.28	47	25.15	18.75	24.5	53	1.39	1.21	0.89	12.8	3.75	30.2	Average

examined cover a wide range of red cell count and hemoglobin content. The counts vary from 0.74 million to 3.33 millions per c.m., and the hemoglobin varies from 3.83 to 12.20 gm. per hundred cubic centimeter. The volume per cent. of red cells as determined by the hematocrit method is uniformly high. The volume index ranges from 1.17 to 1.77. The average is 1.39. The color index is below 1.00 in only three instances and is never higher than the volume index. The saturation index is as high as 1.00 in only six of the twenty cases and is never over 1.00 beyond the limits of error.

The cause of the high color index in pernicious anemia is still not clear in the minds of many. I find on inquiry that most physicians

believe that there is a supersaturation of the cells with hemoglobin. Although Capps pointed out that the color index is never higher than the volume index, and the high color index is thus due to the increase in cell volume, this fact is not made clear in even the most recent text books. The term hyperchromemia is frequently used in the sense of a

TABLE 6.—HEMOLYTIC SECONDARY ANEMIA

R. B. C. in Millions per C.Mm.	Oxygen Capacity per 100 C.c.	Gm. Hb. per 100 C.c.	Hb. in per Cent.	Oxygen Capacity per 100 C.c. per 5 Million Cells	Hemoglobin in Gm. per 100 C.c. per 5 Million Cells	Hematocrit Reading	Volume per Cent. R. B. C.	Volume Index	Color Index	Saturation Index	Volume Average Cell in C.c. × 10-11	Gm. Hb. in Average cell × 10-11	Actual Percentage Hb. in cell	Diagnosis
1.60	5.62	4.20	27	17.55	13.10	17.5	28	1.04	0.91	0.71	9.6	2.62	24.1	Chronic nephritis
1.96	7.25	5.40	35	18.50	13.80	18.0	39	1.00	0.90	0.90	9.2	2.76	30.5	Carcinoma of stomach
2.67	9.25	6.90	44	17.30	12.90	24.0	52	0.98	0.82	0.85	9.0	2.58	28.8	Chronic bronchitis
2.85	8.22	6.13	39	13.95	10.40	25.0	54	0.95	0.68	0.72	8.7	2.08	24.4	Carcinoma of cervix
2.94	5.62	4.20	27	9.58	7.15	22.0	47	0.79	0.46	0.57	7.2	1.43	19.7	Undetermined
3.10	8.98	6.70	43	14.63	10.90	23.0	50	0.70	0.80	0.88	6.4	2.18	29.9	Diffuse carcinomatosis
3.21	10.90	8.14	52	16.81	12.55	30.0	65	1.01	0.81	0.80	9.2	2.51	27.2	Lead poisoning
3.31	10.28	7.87	50	15.78	11.85	28.0	61	0.92	0.76	0.83	8.5	2.37	28.2	Carcinoma of stomach
3.35	12.32	9.15	59	18.15	13.55	31.0	68	1.03	0.88	0.87	9.5	2.71	29.5	Acute endocarditis
3.36	9.04	6.75	43	13.48	10.05	24.0	52	0.78	0.84	0.83	7.2	2.01	28.2	Chronic nephritis
3.36	13.65	10.20	65	20.30	15.15	31.0	68	1.01	0.97	0.96	9.3	3.06	32.6	Pelvic inflammatory disease
3.54	12.80	11.03	71	20.85	15.55	33.5	73	1.02	1.00	0.96	9.4	3.11	33.2	Chronic nephritis
3.63	16.50	12.20	79	22.68	16.95	32.5	71	0.96	0.92	1.04	7.2	3.39	35.3	Malnutrition
3.93	15.80	11.77	75	20.10	15.00	38.0	82	1.05	0.95	0.90	9.7	3.00	30.5	Undetermined
4.03	16.30	12.15	78	20.25	15.10	37.0	81	1.00	0.98	0.96	9.2	3.02	32.6	Obstruction common bile duct
4.10	9.05	6.75	43	11.08	8.25	29.0	62	0.77	0.52	0.68	7.1	1.65	23.1	Wassermann 4+; luetic ?
4.11	17.15	12.82	82	20.85	15.55	39.0	85	1.04	1.00	0.96	9.6	3.11	32.6	Carcinoma of colon
4.15	12.82	9.55	61	15.40	11.50	32.0	69	0.83	0.74	0.89	7.6	2.30	30.2	Pelvic inflammatory disease
4.60	17.60	13.15	84	19.30	14.40	43.0	94	1.02	0.91	0.92	9.4	2.88	31.2	Chronic focal infection
4.61	16.85	12.58	80	18.15	13.55	44.0	95	1.03	0.87	0.85	9.5	2.71	28.8	Mild chlorosis
4.72	16.83	12.66	80	17.82	13.30	44.0	95	1.00	0.85	0.85	9.2	2.66	28.8	Chronic bronchitis
4.99	11.75	8.77	56	11.70	8.80	34.0	75	0.75	0.56	0.71	6.9	1.76	24.1	General gland enlargement
5.00	16.75	12.48	80	16.75	12.50	42.0	92	0.92	0.80	0.87	8.5	2.50	29.5	Catarrhal jaundice
5.04	14.41	14.47	93	19.22	14.35	45.0	98	0.96	0.93	0.96	9.0	2.87	32.2	Conv. acute tonsillitis
5.06	17.67	13.30	85	17.41	13.00	44.0	96	0.95	0.84	0.89	8.7	2.60	30.4	Acetanilid poisoning
5.10	11.10	8.28	53	11.10	8.30	32.0	70	0.66	0.52	0.78	6.3	1.66	26.5	Undetermined
3.78	12.75	9.52	61	17.00	12.70	32.0	70	0.93	0.81	0.87	8.6	2.52	29.5	Average

supersaturation. Likewise, Larrabee did not find the volume index constantly higher than the color index. Our series in which exact methods are used verifies the statement of Capps. The saturation index is of value in emphasizing this point, since it is never more than 1.00 and is seldom as high as 1.00. The average percentage of hemoglobin in the cells is 30.2 as compared with a normal of 33.9.

The average cell volume is variable but always greater than the normal. The average of the twenty cases is 12.8×10^{-11}, or 39 per cent. greater than the average normal. The average hemoglobin content of the cell is 3.75×10^{-11} gm., or 21 per cent. greater than normal.

Several cases in this series are of special interest. Case 8 was a typical case of aplastic anemia of four months' duration. The volume index was 1.22 and the color index was 1.16. The last two cases are unusually interesting. One patient has had a remission of six years, the other of ten years. In each instance there is at present a very high color and volume index. Free hydrochloric acid is still absent on gastric analysis. The patients are, however, free of symptoms. These

TABLE 7.—Hemorrhagic Secondary Anemia

R. B. C. in Millions per C.Mm.	Oxygen Capacity per 100 C.c.	Gm. Hb. per 100 C.c.	Hb. in per Cent.	Oxygen Capacity per 100 C.c. per 5 Million Cells	Hemoglobin in Gm. per 100 C.c. per 5 Million Cells	Hematocrit Reading	Volume per Cent. R. B. C.	Volume Index	Color Index	Saturation Index	Volume Average Cell in C.c. × 10^{-11}	Gm. Hb. in Average cell × 10^{-11}	Actual Percentage Hb. in cell	Diagnosis
1.37	3.19	2.37	15	10.50	7.85	9.5	20	0.73	0.59	0.81	6.7	1.73	20.0	Intestinal hemorrhage
3.00	7.74	5.77	37	12.85	9.60	22.0	47	0.78	0.62	0.79	7.2	1.92	21.0	Hemorrhoids; examined 5/24/21
3.38	8.74	6.52	42	12.90	9.65	25.0	54	0.80	0.62	0.78	7.4	1.93	21.0	Same patient; examined 7/5/21
4.95	13.27	9.90	53	13.47	10.05	36.0	78	0.77	0.60	0.75	7.4	1.00	20.4	Hemorrhoids; examined 3/7/22
3.43	7.02	5.23	34	8.92	6.65	21.0	46	0.67	0.50	0.75	6.2	1.53	17.0	Same patient; examined 4/3/22
3.96	9.40	7.02	45	11.85	8.85	28.0	60	0.76	0.57	0.75	7.0	1.77	19.7	Hemorrhoids
3.93	11.97	8.91	57	15.80	11.40	31.0	67	0.75	0.73	0.85	6.9	2.28	24.8	Gastric ulcer
4.50	12.88	9.62	62	14.35	10.70	32.0	70	0.77	0.70	0.90	7.1	2.14	23.8	Carcinoma of cervix
3.57	9.30	6.92	44	13.00	9.70	25.0	55	0.77	0.62	0.80	7.1	1.94	21.0	Average

findings lend weight to the belief of many clinicians that "once pernicious anemia, always pernicious anemia."

Hemolytic Secondary Anemia.—The secondary anemias have been classified roughly into two types. The anemias due to increased blood destruction within the blood vessels are considered as hemolytic, those due to the loss of blood through hemorrhage as hemorrhagic. This is obviously an unsatisfactory classification but serves the present purpose. Twenty-six observations on hemolytic anemia are recorded (Table 6). These show for the most part a volume index of 1.00 or very little less regardless of the extent of the anemia. The cases presenting a low volume index have probably existed for a long time, the low index being due to long continued drain on the bone marrow. The average volume index of this group is 0.93, the color index, 0.81, and the saturation index, 0.87.

The volume of the average cell in the average of the series is 8.6×10^{-11} c.c. and the average hemoglobin content is 2.5×10^{-11} gm. It is of interest to note that the saturation index and the actual percentage of hemoglobin in the red cells is very nearly equal that of pernicious anemia.

Capps reported a volume index greater than 1.00 in certain cases of jaundice. I have examined the blood of two persons with this condition. In one case the jaundice was due to a catarrhal cholangitis, in the other to an obstruction of the common bile duct by a carcinoma of the head of the pancreas. The jaundice was most intense in both instances. The volume index is 1.00 in one, and 0.92 in the other. Several cases of carcinoma are recorded, including two involving the stomach. The volume index is 1.00 or less.

Hemorrhagic Secondary Anemia.—Only eight cases were studied in this group. The results are tabulated in Table 7. These patients had all suffered from chronic hemorrhage. The volume index is low in every instance, the average being 0.77. The color index is still lower, namely 0.62. The saturation index is higher than either the color or volume index, the average being 0.80. In the final average the cell volume is 7.1×10^{-11} c.c. The hemoglobin content of the average cell is 1.94×10^{-11} gm. The actual percentage of hemoglobin in the red cells is 27.1. No opportunity has presented itself to study cases of acute hemorrhage. It is apparent that in anemia due to very acute hemorrhage there could be no change in volume index. In the cases reported the very low volume of the average red cell is probably the best index of the drain on the bone marrow.

DISCUSSION

The results obtained in this study emphasize in a striking way the great constancy of the volume and hemoglobin content of the normal cell. No variations greater than the limits of error incident to the determination have been encountered. The fact that the hemoglobin in normal blood never exceeds a concentration of 33.9 per cent. can be accepted as the strongest evidence that this figure represents the saturation of the red cells by hemoglobin.

In pernicious anemia the one constant and characteristic feature of the blood is the increased volume of the average red cell. This increased volume has not so far been found in any other condition and it would seem that a volume index beyond the limits of error of the determination is almost diagnostic of pernicious anemia. A color index greater than 1.00 means only that the cells are larger than normal. The volume index of more than 1.00 is always present if the color index

is greater than 1.00 and also in many instances in which the color index is less than 1.00. It is obvious that a knowledge of the volume index is of greater value than the color index in differential diagnosis of the different types of anemia.

The facts presented on the exact volume and hemoglobin content of the average cell in pernicious anemia expressed in absolute instead of relative terms prove beyond question that supersaturation of red

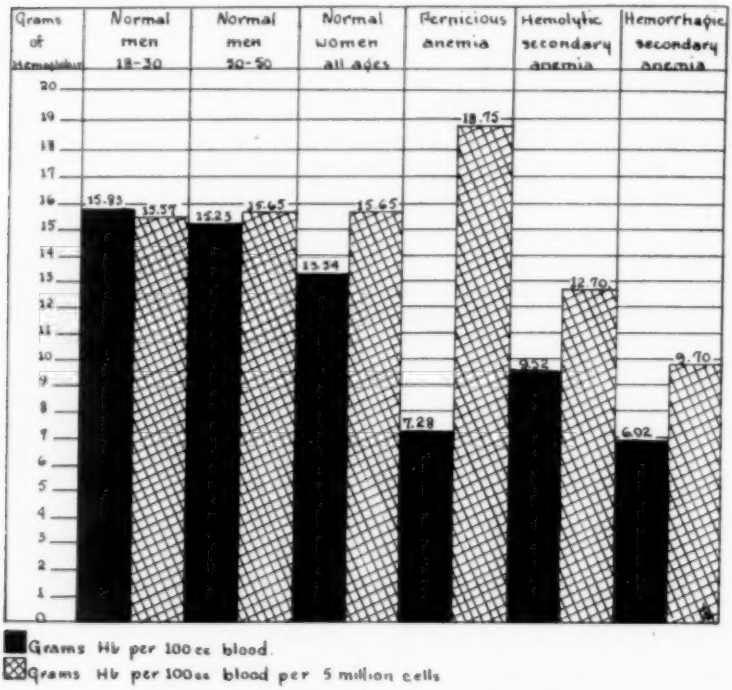


Fig. 1.—Hemoglobin percentage of blood of normal persons and in cases of anemia.

cells never occurs. This is most graphically shown in the single case by the calculation of the saturation index which expresses the amount of hemoglobin in the cell relative to normal, and in every case is 100, or less.

A variation in volume of the red cells is very much more easily determined than a variation in the diameter of the cell. A relatively small increase in diameter causes a very large increase in cell volume.

This is illustrated in Figure 2. To give an increase of 39 per cent. in the volume of the cells it is only necessary to increase the diameter of every cell from the normal 7.60 microns, to 8.48 microns. If the increase is uniform, involving all the cells, as is not uncommonly the case, it is most difficult to detect the increase in size of the red cells by simply looking at them in a fresh preparation or on a stained film.

Price-Jones [12] thinks that there is a diurnal variation in size of the cells. His claim is based on results obtained by measuring the diameter of the cells. The variation in diameters which he describes would make a very large difference in volume. I have made determinations of the

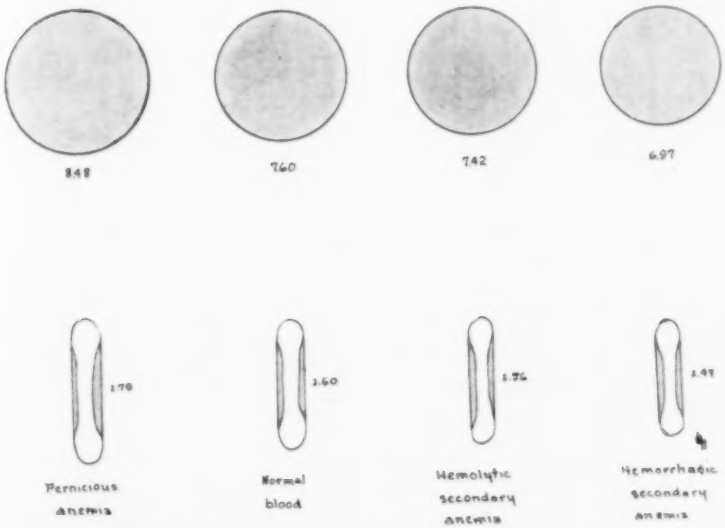


Fig. 2.—Diagram to illustrate the relative measurements of the red blood cell in the various anemias as compared with the normal.

cell volume by the method described at all times of the day and find no evidence of a diurnal variation.

As contrasted with primary anemia, the secondary anemias show an average cell volume never greater than normal and seldom as large. The color index is correspondingly smaller. The saturation index, however, is nearly as great as that in pernicious anemia. The per cent. of saturation of the hemoglobin in the protoplasm probably plays a large part in its ability to transport oxygen. This preservation of concentration is perhaps a protective phenomenon. The smaller size

12. Price-Jones, C.: J. Path. & Bacteriol. **23**:371, 1920.

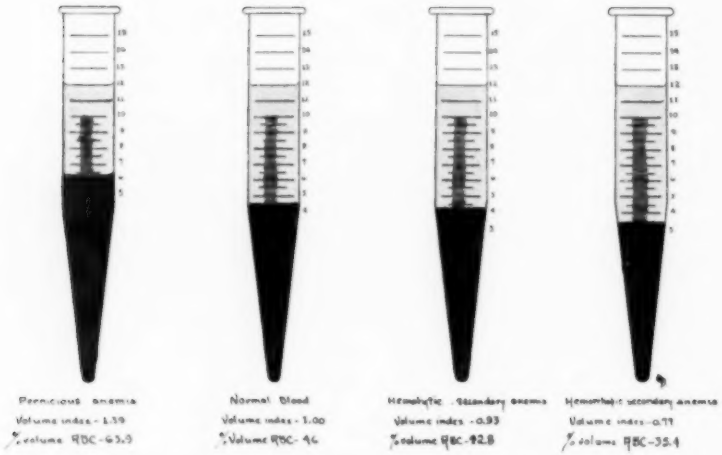


Fig. 3.—Diagram to illustrate the volume of packed red corpuscles obtained on centrifuging 10 c.c. of blood containing 5 million red cells per cubic millimeter.

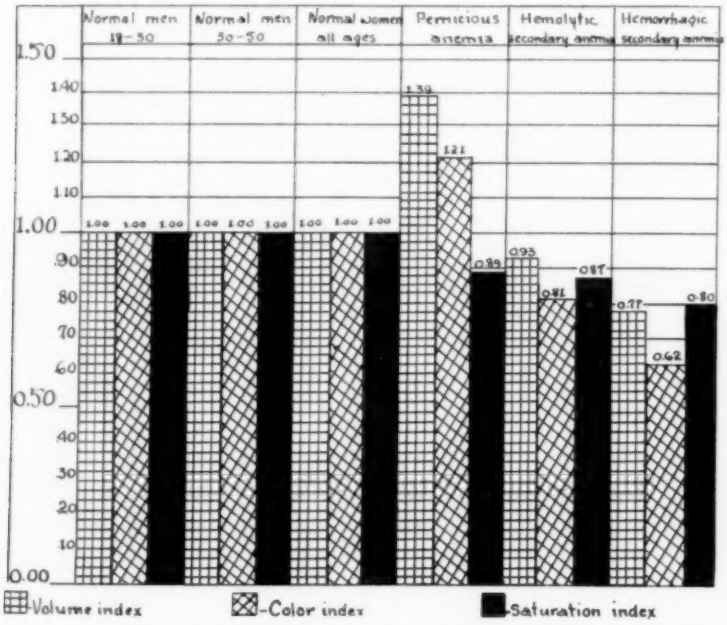


Fig. 4.—Diagram to show the volume, color, and saturation index in normal blood and in the anemias.

of the cell in hemorrhagic anemia is probably the direct result and an expression of the long continued drain on the bone marrow.

The best comparison of the difference in cell mass in different types of blood is best shown by the actual volume of cells obtained on centrifuging a certain amount of blood. If we suppose that the blood from different types of anemias were concentrated so that there would be 5,000,000 cells per cubic centimeter as in the normal, and 10 c.c. of each were centrifuged, the cell volume would be as illustrated in Figure 3. The figures given represent the average of the cells in different groups studied. It will be noted that the cell volume in pernicious anemia is almost twice that of hemorrhagic secondary anemia.

The relation of the three indices in all the groups is illustrated in Figure 4. The indices are equal only in normal blood.

TABLE 8.—Comparison of Average Findings in Normal Blood and in the Anemias

R. B. C. in Millions per C.Mm.	Oxygen Capacity per 100 C.c.	Gm. Hb. per 100 C.c.	Hb. in per Cent.	Oxygen Capacity per 100 C.c. per 5 Million Cells	Hemoglobin in Gm. per 100 C.c. per 5 Million Cells	Hematocrit Reading	Volume per Cent. R. B. C.	Volume Index	Color Index	Saturation Index	Volume Average Cell in C.c. × 10^{-14}	Gm. Hb. in Average cell × 10^{-11}	Actual Percentage Hb. in cell	
5.08	21.30	15.83	101	20.85	15.57	46.5	101	1.00	1.00	1.00	9.2	3.11	33.9	Average men 18-30
4.865	20.35	15.23	98	21.00	15.65	45.0	98	1.00	1.00	1.00	9.2	3.13	33.9	Average men 30-50
4.26	17.33	13.34	85	21.00	15.65	39.0	85	1.00	1.00	1.00	9.2	3.13	33.9	Average women all ages
4.74	19.86	14.80	95	20.95	15.62	43.5	95	1.00	1.00	1.00	9.2	3.12	33.9	Average all men and women
1.94	9.75	7.28	47	25.15	18.75	24.5	53	1.39	1.21	0.89	12.8	3.75	30.2	Average pernicious anemia
3.78	12.75	9.52	61	17.00	12.70	32.0	70	0.93	0.81	0.87	8.6	2.52	29.5	Average hemolytic secondary anemia
3.57	9.30	6.92	44	13.00	9.70	25.0	55	0.77	0.62	0.80	7.1	1.94	27.1	Average hemorrhagic secondary anemia

SUMMARY AND CONCLUSIONS

A study is presented of the red cell count, hemoglobin in grams per hundred cubic centimeters, volume index, saturation index, the exact volume and the exact hemoglobin content, and the hemoglobin percentage in the red cells of the blood of fifty-two normal men and women, twenty cases of pernicious anemia, twenty-six cases of hemolytic secondary anemia, and eight cases of hemorrhagic anemia.

A new index which expresses the hemoglobin saturation of the red cells as compared with the normal is described. In normal blood the red cells are completely saturated with hemoglobin, hence the index is always 1.00.

A study of the volume of the cells is of the greatest value in the differential diagnosis of the anemias. The volume is best expressed as the volume index of Capps.

When determined by exact and absolute methods the volume index, the color index, and the saturation index of normal blood are always 1.00 within the limits of error.

In normal blood the volume of the average red cell is 9.2×10^{-11} gm., and the percentage of hemoglobin in the red cells is 33.9.

In pernicious anemia the practically constant and the most characteristic blood findings is the large volume of the average red cell, as shown by a volume index above 1.00.

A volume index greater than 1.00 has not been found in any condition other than pernicious anemia.

In pernicious anemia the increase in volume of the cell is greater than the increase in hemoglobin. The volume index is consequently always greater than the color index, and the saturation index is never above 1.00 and is seldom as high as 1.00. Supersaturation of the cells by hemoglobin never occurs.

In the twenty cases of pernicious anemia studied, the average volume index is 1.39, the color index 1.21, and the saturation index 0.89. The volume of the cell is 12.8×10^{-11}, the grams of the hemoglobin 3.75×10^{-11} and the percentage of hemoglobin in the cell is 30.2.

In hemolytic secondary anemia the volume of the cell is very little decreased unless there is prolonged drain on the bone marrow, hence the volume index is nearly 1.00. Likewise the color and saturation indices vary only a little from the normal.

In the twenty-six cases of hemolytic secondary anemia studied, the average volume index is 0.93, the color index 0.81, and the saturation index 0.87. The volume of the cell is 8.6×10^{-11} c.c., the hemoglobin content is $2.52 \times ^{-11}$ gm., and the percentage of hemoglobin is 29.5.

The red cells in chronic hemorrhagic secondary anemia have a smaller volume than normal. The extent of the decrease is probably the best index of the drain on the bone marrow and is of definite prognostic value.

Only eight cases of hemorrhagic secondary anemia have been studied. The average volume index is 0.77, the color index 0.62, and the saturation index 0.80. The volume of the average cell is 7.1×10^{-11}, the hemoglobin content is 1.94×10^{-11} gm., and the percentage of hemoglobin in the cells is 27.1.

The percentage of hemoglobin in the cells is very nearly as high per unit volume of cells in secondary anemia as in primary anemia. The difference is in the size of the cells in the two conditions.

BOOK REVIEWS

LEHRBUCH DER PATHOLOGISCHEN PHYSIOLOGIE FUR STUDENTEN UND AERZTE. Herausgegeben von Prof. Dr. H. Ludke, und Prof. Dr. C. Schlayer. Paper. Price, 540 marks. Pp. 819, with 133 illustrations. Leipzig: Johann Ambrosius Barth, 1922.

Under the title mentioned are collected chapters by representative German authors on a variety of physiologic subjects, with discussions of the related functional pathology. Franz Fischler has written the section on metabolism. He mentions methods for studying metabolism, the nitrogen balance, caloric values of food substances, and discusses the importance of various organs, such as the liver, pancreas, and other organs which regulate the normal metabolism. While this section contains much useful information, there is little reference to investigations completed during the last ten years. No mention is made of basal metabolism, or of the work by Du Bois and his associates, neither is the buffer mechanism of the body fluids discussed in any way.

A brief discussion on constitutional pathologic physiology is written by R. v. d. Velden.

Hans Eppinger's chapters on the glands of internal secretion are well written, and his literature references indicate a wide and thoroughly modern conception of facts known in this hazy field of medical biology.

H. Lüdke contributes the section on infection and immunity. Portals of bacterial invasion, defenses of the body, virulence of bacteria, bacterial toxins, natural and acquired immunity and other related features of this topic are discussed. Anything particularly new is not contained, for practically all of the information mentioned has appeared in texts five or more years ago.

Erich Leschke has written the chapters on fever—a review of features generally known and presented well.

The physiology of the nervous system is written by E. Forster, that of the circulation by C. J. Rothberger, of the respiration by Joseph Forschbach, of the blood and blood forming organs (including pathologic physiology) by Hans Hirschfield, of digestion by J. Strasburger, of nutrition by L. Lichtwitz, and of the kidney by C. R. Schlayer. Each of these sections contains an extensive discussion on a par with similar chapters in standard American medical physiologies.

The book contains, in brief, considerable information regarding the subjects discussed. It cannot be recommended to physicians generally as a model text, for practically all the information contained is in standard physiologies and in texts on bacteriology or immunity.

THE CHEMISTRY OF TUBERCULOSIS. A Compilation and Critical Review of Existing Knowledge on the Chemistry of the Tubercle Bacillus and Its Products; The Chemical Changes and Processes in the Host; The Chemical Aspects of the Treatment of Tuberculosis. By H. Gideon Wells, M.D., Ph.D., Professor of Pathology in the University of Chicago, Lydia M. DeWitt, M.D., A.M., Associate Professor of Pathology in the University of Chicago, and Esmond R. Long, Ph.D., Assistant Professor of Pathology in the University of Chicago. Cloth. Price, $5. Pp. 447. Baltimore: Williams & Wilkins Company, 1923.

The increasing activity in research medicine in America becomes apparent in the number of monographs published during recent years. This most recent addition to the list summarizes the careful reading as well as the more recent

work of the members of the Sprague Institute interested more particularly in tuberculosis. The publishers state that it is a complete reference handbook on the chemical aspects of tuberculosis and related problems. It is that. The material is quite recent, the presentation is well balanced, and seemingly nothing of importance has escaped consideration. It is invaluable to the worker in tuberculosis.

It is certainly refreshing and perhaps significant to note that the term "antibody" finds no place in the index. We are apparently advancing in our knowledge of tuberculosis when speculation gives place to accurate observation of biochemical and biophysical processes.

MULTIPLE SCLEROSIS (DISSEMINATED SCLEROSIS). An Investigation by The Association for Research in Nervous and Mental Diseases. Report of the Papers and Discussions at the Meeting of the Association, New York City, December 27th and 28th, 1921. Editorial Board: Charles L. Dana, M.D., Smith Ely Jelliffe, M.D., Henry Alsop Riley, M.D., Frederick Tilney, M.D., Walter Timme, M.D. Cloth. Price, $3.75. Pp. 241, with illustrations. New York: Paul B. Hoeber, 1922.

This book consists of papers on all phases of the subject by twenty-six prominent American investigators of nervous diseases, together with questions and comments by members of the "Commission" of the Association, and conclusions by the commission as a whole. The most interesting chapter is that by G. B. Hassin on the pathology. He considers the lesions as purely degenerative, not inflammatory, a view not shared by some of those present, as seen from the record of the animated discussion. The book can be recommended as setting forth in a novel and fascinating manner all that is known about this peculiar disease, including the results of the most recent researches in many countries.